EVALUATION OF DIAGNOSTIC TESTS IN MALARIA

(EVALUATING THE DIAGNOSTIC VALUE OF QUANTITATIVE BUFFY COAT AND RAPID DIAGNOSTIC TEST USING PLASMODIUM LACTATE DEHYDROGENASE AGAINST THE PERIPHERAL SMEAR MICROSCOPY)

Dissertation submitted to

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In partial fulfilment of the regulations

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INSTITUTE OF CHILD HEALTH AND

HOSPITAL FOR CHILDREN

MADRAS MEDICAL COLLEGE

APRIL 2012

CERTIFICATE

This is to certify that the dissertation titled, **"EVALUATION OF DIAGNOSTIC TESTS IN MALARIA (EVALUATING THE DIAGNOSTIC VALUE OF QUANTITATIVE BUFFY COAT AND RAPID DIAGNOSTIC TEST USING PLASMODIUM LACTATE DEHYDROGENASE AGAINST THE PERIPHERAL SMEAR MICROSCOPY)"** *submitted by* ***Dr.U.Sasireka,*** *to the Faculty of Pediatrics, The Tamilnadu Dr.M.G.R Medical University, Chennai, in partial fulfilment of the requirements for the award of M.D. Degree (Pediatrics) is a bonafide research work carried out by her under our direct supervision and guidance, during the academic year 2009-2012.*

Prof. Dr.V.Kanagasabai,M.D,
Dean,
Madras Medical College,
Chennai – 600003.

Prof.Dr.P.Jeyachandran,M.D.,DCH
Director and Superintendent,
Institute of Child Health and
Hospital for Children,
Chennai - 600008.

Dr.S.SUNDARI,M.D.,D.C.H,
Addl.Professor of Pediatrics,
Institute of Child Health
and Hospital for Children,
Chennai - 600 008.

DECLARATION

I, **Dr.U.Sasireka**, solemnly declare that the dissertation titled **"EVALUATION OF DIAGNOSTIC TESTS IN MALARIA (EVALUATING THE DIAGNOSTIC VALUE OF QUANTITATIVE BUFFY COAT AND RAPID DIAGNOSTIC TEST USING PLASMODIUM LACTATE DEHYDROGENASE AGAINST THE PERIPHERAL SMEAR MICROSCOPY)"** has been prepared by me under the expert guidance and supervision of **Dr. S. SUNDARI, M.D, D.C.H.,** Additional Professor, Institute of child health and hospital for children, Madras Medical College, Chennai – 3.This is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of the rules and regulations for the M.D. Degree Examination in Pediatrics (Branch VII).

Place : Chennai

Date : **DR.U.SASIREKA**

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Institute of Child Health and Hospital for Children
MADRAS MEDICAL COLLEGE
Halls Road, Egmore, Chennai - 600 008.
Ph : 28191135 / Direct : 28194181 / Fax : 044 - 28194181.

Date.....

Ref.No.Dir/EC/ICH/07

Institute of Child Health and
Hospital for Children,
Chennai-08.
dated:

The Institutional Review Board [Ethical committee] of Institute of Child Health and Hospital for Children, Chennai-08, was held on 30.01.2010 at 10.00AM at the Deputy Superintendents chamber.

Members Present: Dr.R.Kulandai Kasthuri
Chair Person.

Members:
1. Dr.K.Gita
2. Dr.P.Jeyachandran
3. Dr.D.Vijaya Sekaran
4. Prof.Girija Shyam Sundar
5. Mrs.Muthu Lakshmi, (Advocate)
6. Dr.P.Ramachandran
7. Mrs.Shubha Kumar

Member Secretary: Dr.Luke Ravi Chellaiah

Title: "A Study on Evaluation of diagnostic Tests in Malaria".

The Institutional Review Board was satisfied with the revised format submitted by you. Hence the Institutional Review Board is pleased to approve the study.

To,
Dr.U.Sasirekha,
Post Graduate,
ICH & HC,
Chennai-08.

Director and Superintendent.

CONTENTS

INTRODUCTION

Malaria, called as the King of diseases is caused by Plasmodium infection. It's the most important infectious disease in tropical and sub tropical regions, and continuous to be the major public health problem. Over 40% of the world's population is exposed to the risk of malaria. According to the World Malaria Report 2010, there were 225 million cases of malaria and an estimated 7, 81,000 deaths in 2009, a decrease from 233 million cases and 9,85,000 deaths in 2000. Most deaths occur among children living in Africa, where a child dies every 45 seconds due to malaria and the disease accounts for approximately 20% of all childhood deaths. Children aged one to four are the most vulnerable group to infection and death[1].

Etiology[2]

There are four species of Plasmodium that cause malaria: P.vivax, P.malariae, P.ovale,P.falciparum. P.vivax infection is the most common. Infection by P.falciparum is the most serious,being responsible for deaths among children. The sporozoite is transmitted to the host by anopheline mosquito. Transmission may also occur transplacentaly and rarely through blood transfusion.

Burden of malaria: In India[3]

Burden of malaria in India is 1.49 million, with as many as 0.9 million cases of P. falciparum malaria per year. Malaria causes 2,05,000 deaths per year in India with 55,000 in early childhood, 30,000 at ages 5-14 years. About 90 per cent of the deaths were recorded in rural areas. The maximum number of cases were reported in Orissa (50,000 cases) followed by Chattisgarh, Jharkhand and Assam.

Malaria in Tamilnadu[4]

Out of the total malaria cases in our state, two thirds occurring in Chennai.

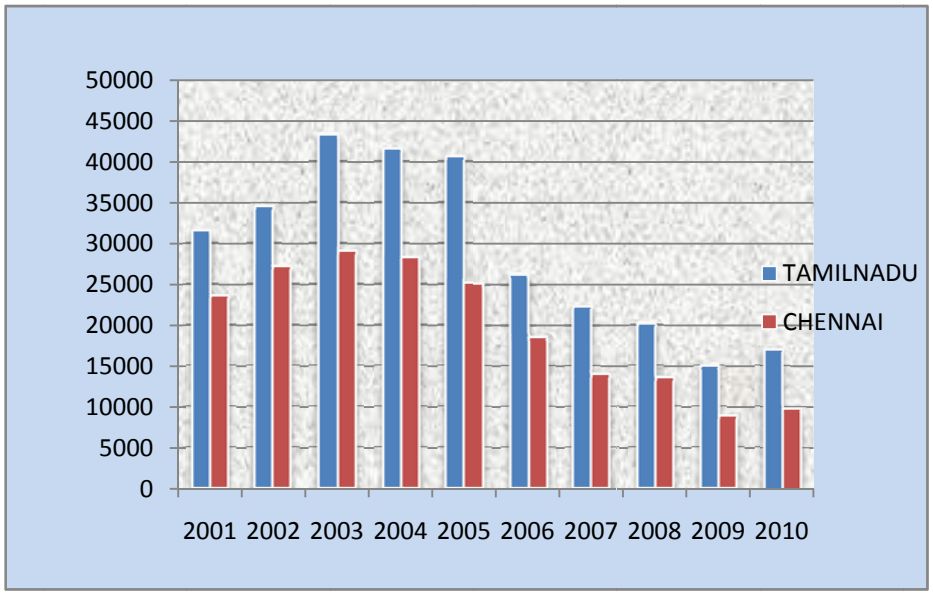


Fig1. Showing the incidence of malaria cases in Tamilnadu and the cases contributed by chennai.

Table 1: Malaria cases in Tamilnadu.

Year	State	Rural	Chennai	Chennai %	Other ums	Other ums %
2001	31551	5121	23652	75.0	2778	8.8
2002	34523	5490	27205	78.8	1828	5.3
2003	43396	12233	29058	67.0	2105	4.9
2004	41640	10841	28229	67.8	2570	6.2
2005	40594	13560	25153	62.0	1881	4.6
2006	26329	6529	18585	70.6	1235	4.7
2007	22389	7104	14002	62.5	1283	5.7
2008	20211	5737	13503	66.8	971	4.8
2009	14920	4274	8917	59.8	1729	11.6
2010	17062	6002	9789	57.4	1271	7.41

Malaria Burden in Institute of Child Health and Hospital for Children

Malaria cases reported in Institute of Child Health and Hospital for Children during the year 2010 was 564.Among them 14 cases were diagnosed to have P.falciparum infection.

Fig 2. Total Malaria cases in ICH

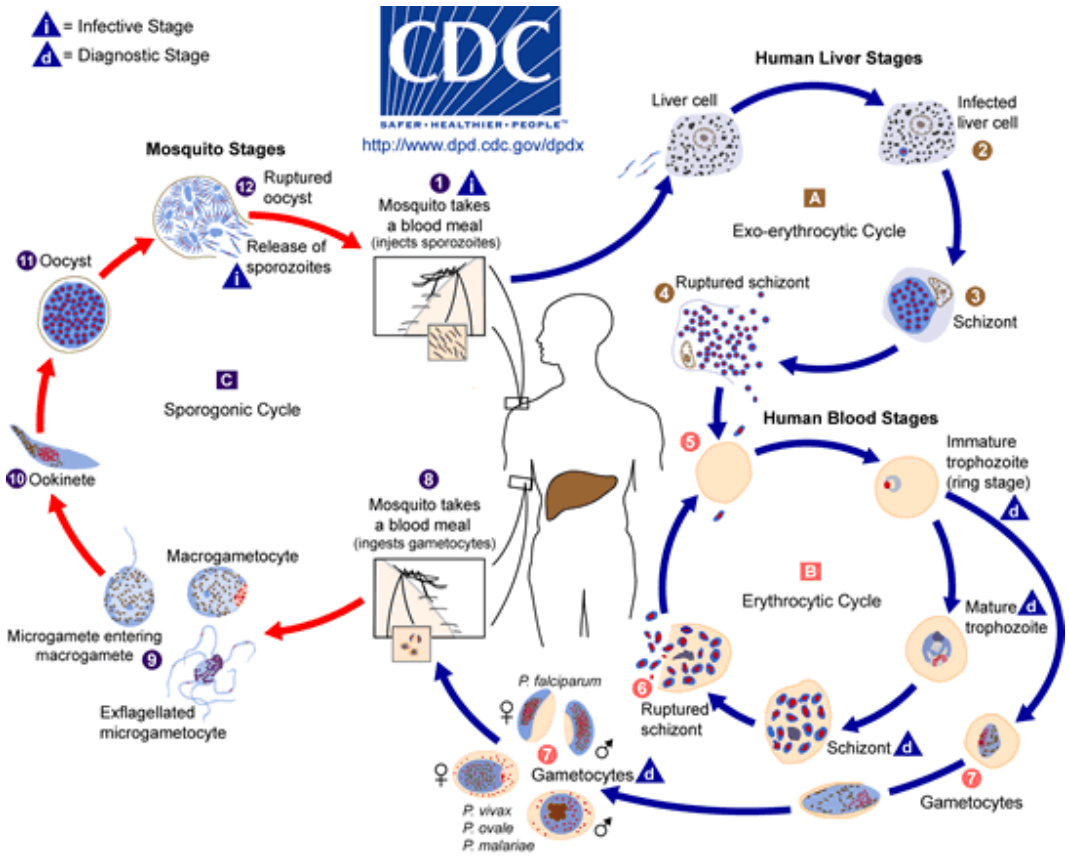
Table 2: Malaria in Institute of Child Health and Hospital for Children from 2001 to 2010

Year	Total malaria cases	P.falciparum cases	Total Deaths
2001	497	6	5
2002	607	12	5
2003	517	13	1
2004	401	11	3
2005	313	16	3
2006	318	15	2
2007	440	13	4
2008	596	19	6
2009	556	10	5
2010	571	14	0

The magnitude of the problem is further enhanced by P.falciparum resistance to standard antimalarial drugs, adding to increased mortality and morbidity[5]. Hence our efforts should be directed towards more restrictive use of the drugs and uniform prescribing practices to limit the spread and intensification of drug resistance. Remarkable decrease in antimalarial drug use could be achieved by improving the diagnosis of malaria.

Though microscopy is considered as the gold standard method for diagnosing malaria, it is time consuming, labour intensive and requires considerable expertise for it's interpretation, and variable sensitivity and specificity compared to the recent technical advances, particularly at low levels of parasitemia. Majority of malaria cases were contributed by the developing countries, where cost effectiveness is very much important. The urgency of obtaining results with suspected acute malaria makes some of the sensitive methods for diagnosis of malaria highly difficult. Availability of a rapid, simple and accurate test could greatly aid in the early diagnosis of malaria including in remote areas, where health facility coverage is low. So, we are evaluating the diagnostic value of QBC and RDT (pLDH) in comparison with the gold standard peripheral smear microscopy for diagnosis of malaria in children.

LIFE CYCLE OF MALARIA



Life cycle of malaria [6]

During a blood meal, a malaria-infected mosquito inoculates sporozoites into the human host (1),which infect liver cells (2)and mature into schizonts (3).This schizont rupture and release merozoites(4). This initial replication in the liver is called as (exo-erythrocytic schizogony-A). Then the parasites undergo asexual multiplication in the Red Blood Cells (erythrocytic schizogony- B). Merozoites infect red blood cells (5).The ring stage trophozoites mature into schizonts, which inturn rupture and release merozoites (6). Some parasites differentiate into sexual erythrocytic stages –gametocytes (7). Erythrocytic schizogony is responsible for the clinical manifestations of malaria. The male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal (8). The multiplication of the parasite in the mosquito is known as the sporogonic cycle (c).In the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes (9).The zygotes in turn become motile ookinetes – (10).Ookinetes invade the midgut wall of the parasite and they develop into oocysts (11).The oocysts rupture and release sporozoites (13),which reaches the salivary glands of the mosquito. Inoculation of the sporozoites (14) into a new human host, leads on t continuation of life cycle.

Diagnosis of malaria

Clinical diagnosis[7]

The classic presentation of malaria consists of paroxysms of fever alternating with periods of fatigue but otherwise relative wellness. Febrile paroxysms are characterized by high fever, rigors, sweats, and headache, as well as myalgia, back pain, abdominal pain, nausea, vomiting, diarrhoea, jaundice, splenomegaly, hepatomegaly, anemia, thrombocytopenia, a normal or low leukocyte count, or any combination of these manifestations. Paroxysms coincide with the rupture of schizonts that occurs every 48 hrs with P. vivax and P. ovale and result in every other day fever spikes. Rupture of schizonts occurs every 72 hrs with P. malariae and results in fever spikes every 3rd or 4th day. Periodicity is less apparent with P. falciparum and mixed infections.

Severe, high-risk malaria is characterized by a depressed level of consciousness, seizures, irregular respirations or airway obstruction, hypoxia, hypotension, tachycardia, dehydration, hypoglycemia, metabolic acidosis, and hyperkalemia.

A clinical diagnosis of malaria is still challenging because of the vague signs and symptoms, which overlaps with other common infections. The overlapping of malaria symptoms with other tropical

diseases impairs diagnostic specificity, which will lead on to the indiscriminate use of anti malarials and compromise the care for patients with non malarial fevers.

Laboratory diagnosis

Peripheral smear (thick & thin smear) [8,9]

Peripheral smear (Thick and thin smear) microscopy is considered to be the gold standard test for diagnosing malaria. Although the expert microscopist can detect upto 5 parasites /µl, the average microscopist detects only 50-100 parasites /µl [10,11.] The efficiency of the test depends on various factors like equipment; quality of the smear and the most important, the skill of the technician .The minimum time required for smear examination including preparation of smear will be 20 to 60 minutes.

Thick smear:

For preparing thick smear 2 -3 drops of blood are put on slide. With the corner of the slide and by circular motion the blood drops are mixed and spread over an area of 2 cm. It is dried for 30 min. The red blood cells will be lysed. So the only detectable elements are the malarial parasites (if present), and leukocytes . Then Giemsa's stain is used for

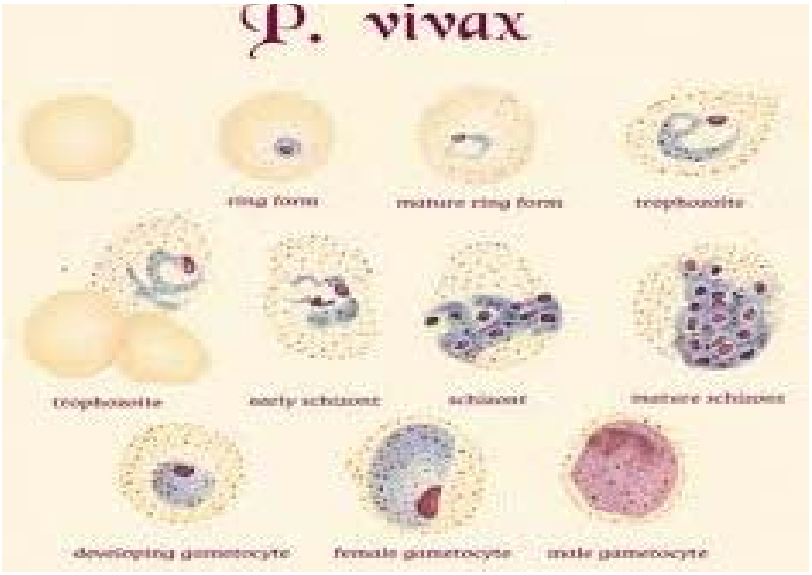
staining the smear and microscopic examination is done. Thick smear is used to detect the presence of infection, and it is also used to estimate parasite concentration. 200 leucocytes are counted in 100 fields (0.25 µl of blood).The parasites are counted against WBCs. If >10 parasites are counted, then the following formula can be applied: (Number of Parasites/ Number of WBCs counted) x 8000 = Number of parasites/µl. A minimum of 200 oil immersion fields should be examined in the thick film [12,13,14].The sensitivity of the thick smear is 5 to 10 parasites/µl.

If malarial parasites are detected in a thick film, then the thin film should be examined to identify the species. If an observer is uncertain whether malaria parasites are present in a thick film, an entire thin film should be examined with a x 100 objective, starting with the edges and the tail where parasitised cells may be more frequent.

Thin smear:

A drop of blood is evenly spread into a thin layer by using the edge of another slide. A good smear should be one blood cell in thickness. The smear is air dried for 10 minutes and is fixed in methanol. Staining is done with Giemsa's [15] ,Wright's or Leishman's stain. Then the smear is examined.

PERIPHERAL SMEAR: PLASMODIUM VIVAX



Diagnostic Points

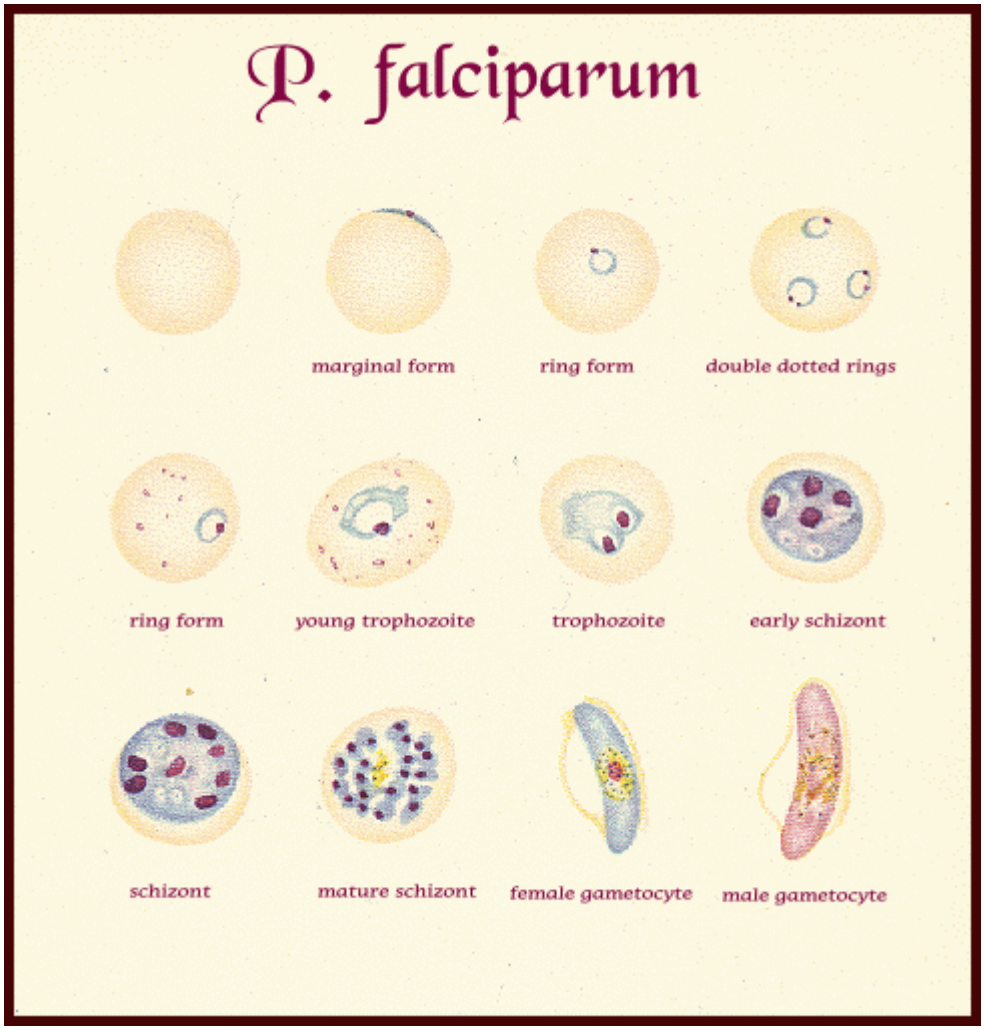
1. Red cells containing parasites are usually enlarged.

2. Schuffner's dots are frequently present in the red cells as shown above.

3. The mature ring forms tend to be large and coarse.

4. Developing forms are frequently present.

PERIPHERAL SMEAR: PLASMODIUM FALCIPARUM



Diagnostic Points

1. Red Cells are not enlarged.
2. Rings appear fine and delicate and there may be several in one cell.
3. Some rings may have two chromatin dots.
4. Presence of marginal or applique forms.
5. It is unusual to see developing forms in peripheral blood films.
6. Gametocytes have a characteristic crescent shape appearance. However, they do not usually appear in the blood for the first four weeks of infection.
7. Maurer's dots may be present.

Totally 1000 RBCs are counted, and among them parasitized RBCs are noted. The percentage of parasitized RBCs is calculated by the following formula: (parasitised RBCs/1000)*100. Determining the percentage of parasitaemia is essential for P. falciparum. Species identification is much easier using thin films.[16] The sensitivity of thin smear for the detection of parasites is low (200 parasites/µl).

Advantages

1. Provides a permanent record of the sample so that it can be re-examined as often as necessary.
2. Very informative : parasites can be characterized in terms of species and life cycle stages. More over the parasite densities can be quantified. Such quantification are helpful to demonstrate hyper parasitemia and to assess parasitological response treatment.
3. Relatively inexpensive,cost estimates are around 4-5 rupees per slide in field conditions.

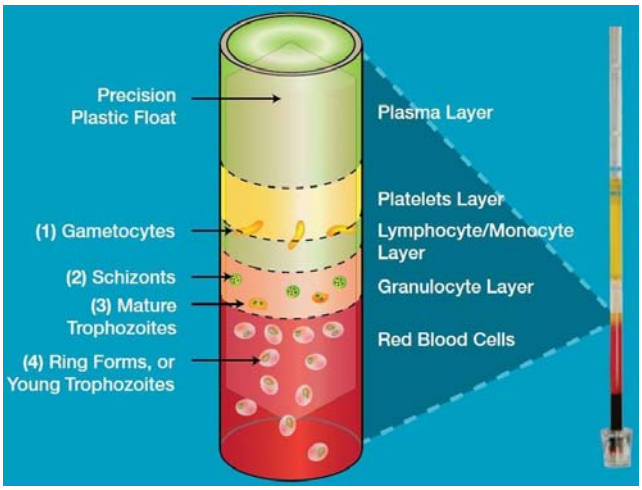
Disadvantages

1. Dependence on good techniques, quality reagents and microscopes, and well trained technicians.
2. Diagnosis of malaria infection with low parasitemia (sequestered parasites of P. falciparum) and to report a negative smear by the thick smear, it requires extended examination.
3. There are often long delays in providing the microscopy results to the clinicians, so that decisions on treatment are commonly taken without the benefit of the results.

Quantitative Buffy Coat (QBC) [17]

The Quantitative Buffy Coat technique (QBC) employs microhematocrit centrifugation which is an effective means of concealing malarial parasites prior to direct examination. It employs a precisely constructed capillary tube which is internally coated with EDTA and acridine orange. The other fluorochromes used are benzothiocarboxypurine (BCP) and Rhodamine-123. The infected red cells appear to be less dense than uninfected ones, and concentrate primarily within the zone near the top of the RBC column[18] .The insertion of a plastic float prior to centrifugation ,concentrates the parasites. The float serves to expand the buffy coat by creating a 40 µ wide area within

QUANTITATIVE BUFFY COAT TECHNIQUE



the buffy layer defined by the exterior of the float and the interior of the capillet. Fluorescent dyes have a special affinity for the nucleic acid in the Plasmodium and will attach to it. When excited by UV light at an appropriate wave length (490 nm), the nucleus will fluoresce apple green. Morphologic characteristics can be examined by fluorescence microscopy, since nuclei fluoresce bright green. When examination cannot be carried out right away, the parasites retain their morphology over several days at room temperature or for at least 7 days when stored in the refrigerator. Malarial parasites thus confined to the periphery of the tube can be counted in this system using a light microscope, although fluorescence is essential for speciation. As a consequence of displacement and concentration, almost all parasites collected in the tube are visible. The ability in identification of species ranges from 75% to 93%.

Quantification of parasite:

1+ < 1 parasite per QBC field

2+ 1-10 parasites per QBC field

3+ 11-100 parasites per QBC field

4+ > 100 parasites per QBC field

Table 3: Features differentiating P.falciparum & P.Vivax

P. falciparum	P. vivax
Can infect all stages of RBCs and, therefore, typically will be distributed equally throughout the packed RBC layer	Infects reticulocytes, which represent a small portion of blood cells and so usually found predominantly in the top of the packed RBC layer
Greater ratios of immature trophozoites to mature trophozoites and schizonts	Smaller ratio of immature trophozoites to mature trophozoites and schizonts
Double infections, i.e. two trophozoites in one RBC	Single infections
Yellow-green crescent, or sausage-shaped gametocytes	Round gametocytes

Advantages

1. Useful for screening large number of samples.
2. Quick because parasites concentrated in narrow zone where they can be viewed with ease. Over 100 tubes screened with ease in 4 hours.
3. Parasites may be detected 12 - 48 hours before visible on thick smears.
4. Increased sensitivity when compared to thick film analysis. It can detect as low as 5-10 parasites/µl of blood[19].

Disadvantages

1. Requires specialized instrumentation, which is more costlier than conventional light microscopy.
2. Poor at determining species.
3. The nuclear remnants, howeljolly bodies can give a false positive result.

Rapid diagnostic tests/ Immunochromatographic tests (RDTs)[20]

RDTs are lateral flow immuno-chromatographic methods .The antigens commonly used are aldolase, PfHRP2[21], pLDH. The first step of the test procedure involves mixing the patient's blood with a lysing agent in a test strip or well. This ruptures the red blood cells, releasing more parasite protein. Labeled antibody, either in the well or on the strip, may then bind with the target antigen.

The resulting mixture of blood products and antigen-labeled antibody complex then passes along the nitro-cellulose strip, over the test and control bands.

The free, labeled antibody will capture the parasite antigen if present which will in turn be captured by the test-band antibody. The

accumulation of microscopic dye particles on the thin band produces a visible line if sufficient antigen-labeled antibody complex is present.

The control band will become visible as sufficient labeled antibody accumulates on the line. Antibodies (or antigen) bound to the strip captures labeled antibodies which failed to bind to antigen from the patient's blood. A visible control line indicates that labeled antibody has traversed the full length of the strip, past the test line.

Advantages

1. Easy to perform and interpretation is simple enough to be grasped even by an illiterate. There is little difference in interpretation among individual users.
2. RDTs do not need any special equipment or training. Technique can be learnt within few hours with good retention of skills.
3. Rapid method , can be performed in 5-15 minutes .Same day results are possible, resulting in fewer patients lost to follow-up and quicker treatment.
4. The kit has the shelf life of 1-2 years at ambient temperatures, with no need for refrigeration.

5. Because RDTs detect circulating antigens, they are capable of detecting plasmodium falciparum infection when the parasites are sequestered in the deep vascular compartment[22].

Disadvantages

1. Unfortunately, the test strips are not as sensitive as microscopy for detecting low level malaria infections (approximately <100-200 parasites/µl of blood) and cannot quantify the parasite load in the patient. So it has lesser prognostic significance.
2. Kits that detect both P.falciparum & non falciparum species are unable to differentiate between P.vivax, P.ovale & P.malariae
3. Antigen persistence : where the protein detected by the strips circulates in the blood for days after the parasites have been successfully killed by antimalarial drugs. These post-therapeutic false positive readings may be improperly interpreted as the presence of drug-resistant malaria.
4. Cross-reactivity with rheumatoid factor in blood generates a false positive test line, but replacement of IgG with IgM in recent products reduces this problem.[23, 24, 25]

RDT using pLDH

The monoclonal antibodies used in RDT (pLDH) are prepared from infected erythrocytes. They are 6C9,17E4,19G7. Among them 6C9 and 19G7 are pan specific antibodies.17E4 is specific for P.falciparum. When pLDH is present in blood, that will be captured by 6C9 (pan specific antibody) which is conjugated to gold particles that acts as an indicator. The bound antigen-antibody complex will migrate over the nitrocellulose dipstick. The antibodies-17E4 and 19G7 will act as separate immobilized capture sites on the dipstick. The malaria antigen-labelled antibody complex will be captured by either or both of the immobilized capture sites (P. falciparum) or by the pan specific site only (non falciparum species).The successful test is indicated by the presence of a goat anti-mouse monoclonal antibody capture control line. The mixed infection will also be interpreted as P.falciparum. Here the genus specific line will be darker than the species specific line. The monoclonal antibody have been tested for cross-reactivity with LDH from other blood protozoa, pathogenic bacteria and fungi. But there is no evidence of such cross reactivity.[26]

Advantages

pLDH is produced from glycolytic pathway of parasites. The presence of LDH detected by the test indicates that there are viable parasites. So it can be used to follow the decline in parasitaemia during treatment[27]. The other antigens like HRP2 can't be used for this purpose. The samples infected with P. vivax are easily distinguished from those infected with P. falciparum; mixed infections with both P. falciparum and P. vivax can also be detected.[28]

Polymerase Chain Reaction[29,30]

PCR based technique is most specific and sensitive diagnostic method, particularly for malaria cases with low parasitemia and mixed infection.[31] The PCR technique is used extensively to confirm malaria, to follow the therapeutic response, and to identify the drug resistance.[32] It is more sensitive than QBC and some RDTs.[33, 34] PCR has shown higher sensitivity and specificity than peripheral blood smear examination.[35] PCR can detect as low as 1-5 parasites / μl of blood and it can detect drug resistant parasites, mixed infection. It can be used to screen large numbers of samples.[36,37]

LAMP technique

This technique detects the conserved 18S ribosome RNA gene[38]. It has higher sensitivity and specificity for both P.falciparum and non falciparum infections.[39,40]

Other methods

Fluroscent antibody technique

Flow cytometry [41,42,43.]

LITERATURE REVIEW

1. Parija *et al*.,2009 [44] conducted a study in the Department of Microbiology, JIPMER, Pondicherry and compared the efficacy of thick and thin smear, quantitative buffy coat (QBC), RDT(pLDH) in 411 patients presenting with symptoms of malaria. Thick smear was taken as gold standard. Thin smear had a sensitivity of 54.8% and specificity of 100%. Out of 411 samples, QBC detected 66 cases and Malariagen detected 62 cases, with a sensitivity of 78% and 75%, respectively. The conclusion was where facilities are available, QBC should be used for the routine diagnosis of malaria because interpretation of thick smear was difficult in their studies.

2. Narayanappa and his collegues in 2008[45] , compared the sensitivity & specificity of RDT(LDH) & QBC in a prospective study with 103 children. Smear and RDT(pLDH) was positive in 59 patients.QBC was positive in 60 patients.The sensitivity and specificity of QBC for detection of P.falciparum was 100% and 95.8% and for P.vivax it was 91.3% and 98.2% . The sensitivity and specificity of RDT (pLDH) for detection of P.falciparum was 100% and 97.8% and for P.vivax it was 100% and 98.2%. He

concluded that RDT (LDH) test meets the most criteria for an ideal diagnostic test which is simple, rapid, sensitive, specific and easy to perform.

3. Manjunath *et al*., 2011 [46],conducted a prospective study in the Department of Microbiology, Shri BM Patil Medical College , Bijapur, Karnataka in which QBC was compared with thick and thin peripheral smears and malaria antigen test. A total of 387 samples were collected from patients presenting with fever and chills. Malaria was diagnosed in 60, 72 and 56 patients by Leishman staining technique, QBC method and malaria antigen test respectively. The QBC method allowed an additional 12 case. In 315 patients who were negative by the QBC, malaria antigen test and the Leishma stained smears were also negative for malarial parasite.Although the sensitivity of QBC is high,the species identification was not possible in 32 cases. The conclusion was Leishman stained thin blood smear still appear superior for species identification and QBC method has its advantages in terms of speed, sensitivity and ease.

4. Stephens *et al.,*1999 [47] in North west Thailand did a study which compared clinical diagnosis,peripheral smear microscopy and RDT(Parasight-F) in 301 people.Clinical diagnosis of malaria was

made in 204 cases by triage nurses.RDT picked up 158 cases. Based on the microscopy results, they found that a presumptive clinical diagnosis dramatically over-diagnosed malaria, and similarly there were a large number of false positives using the ParaSight-F test.they concluded that many of the patients had received some form of malaria treatment prior to presentation at the hospital, and that the high number of false positives are explained by persistent antigenemia and the possibility of there being sequestered parasites following incomplete chemotherapy.

5. Palmer et al.,1997 [48] examined the ability of RDT using pLDH (OptiMAL) to detect P. vivax and P. falciparum in comparison with thick smear in Northen Honduras during a malaria outbreak in 202 patients of suspected malaria. Out of them 96 (48%) were positive by smear and 91 (45%) were positive with RDT. The sensitivity of RDT for P. falciparum and P. vivax was 94 and 88% respectively. The specificity for P.vivax and P.falciparum 100 and 99%, respectively. RDT (pLDH) missed 3 % of cases in which the parasite level was <100 parasites/µl of blood. Those cases were detected by microscopy.

6. In a field study by Quitana *et al.*,1998[49] P. falciparum and P. vivax parasites were detected by thick-film microscopy with the mean parasite density of 590/mm^3. When compared with thick smear, RDT (pLDH) had a sensitivity of 100% and a specificity of 95% for samples containing P. falciparum.The sensitivity and specificity of the RDT (pLDH) were similar to those of thick smear for P. vivax.

7. Hunt-Cooke *et al.,* 1999[50] conducted a study with 636 samples. The sensitivity and specificity of OptiMAL for the diagnosis of Plasmodium falciparum parasites from untreated patients was 95.3% and 100% respectively. For Plasmodium vivax, the sensitivity was 96%.

8. Iqbal *et al.,* 2001[52] compared RDT (pLDH) (OptiMAL) to microscopy and PCR. In their study half of the patients had <50 parasites/µl and they are not detected by RDT (pLDH). When the parasite levels are >100 parasites/µl , the sensitivity of RDT was 97%. The pLDH assay had an advantage over the ICT Pf assay for HRP-2 in distinguishing P. falciparum from mixed infections with both P. falciparum and P. vivax. [52]

9. Geoffrey *et al*.,2002[53] compared the The ICT P.f/P.v assay with OptiMAL and found that The ICT P.f/P.v assay was highly sensitive (97%) for the diagnosis of clinically significant P. falciparum infection. It demonstrated a low detection threshold, although only two specimens with parasite densities of <100/µl were assessed. However, the assay performed particularly poorly in diagnosing P. vivax malaria (sensitivity, 44%), with false-negative results encountered for infections with densities up to 10,000 parasites/µl. On the other hand, the OptiMal assay demonstrated only moderate sensitivity for both P. falciparum and non-falciparum malaria, generally consistent with previous report.

10. Moody *et al*., 2002[54] compared the smear with RDT (pLDH) , in Department of Clinical Parasitology ,London, In their study the sensitivity of RDT (pLDH) was low for non-P. falciparum malaria other than P. vivax.

11. Fryauff et al., [55] found the sensitivity of RDT(pLDH) for detecting malaria with 500 to 1,000 parasites/µl was 88 to 92% and it is less sensitive in discriminating between malaria species when compared to smear examination.

12. Malik *et al*., [56] analysed QBC, RDT (pLDH) and smear results of 124 blood and found, QBC had a sensitivity and specificity of 94.3% and 97.6%, respectively.RDT (pLDH) had a sensitivity and specificity of 90.4% and 100%, respectively. The RDT (pLDH) failed to identify two Plasmodium vivax infections at parasite counts of 5000/µl and > 200/µl, suggesting that plasmodial gene deletions could be responsible for non-expression of pLDH.

    In several clinical trails, the decline in pLDH activity parallels the decline of viable parasites during therapy [54,57,58,59] .So RDT (pLDH) may be used to monitor the progress during therapy.

13. Nandwani *et al*., 2005[60] conducted a study in Microbiology Department ,University College of Medical Sciences, New Delhi, and found QBC was 97.5% sensitive and 100% specific for detection of all stages and species of malarial parasite. The species identification was easy once the staining was optimised. The QBC test required considerable amount of practice, costly equipment, however it was fast and in our study was found to be highly sensitive.

14. Krishna B .V *et al*., 2003[19] conducted a study in Microbiology Department, Karnatak Institute of Medical Sciences, Hubli with

1435 blood samples compared QBC and smear. QBC detected 57 (3.97%) cases, while only 44 (3.07%) samples were positive by the smear. Among the QBC positive cases, 27 were P.vivax, 26 were P. falciparum cases and mixed infection (P. vivax and P. falciparum) was observed of in 4 cases. Samples with low parasitaemia (QBC grades 1+ and 2+) were often found to be negative by blood film examination. Conclusion was QBC method is easy to perform, had a higher sensitivity.

15. Pinto *et al*., 2001 [61] compared thick flim and QBC.The study was conducted in Department of Microbiology, Goa Medical College with 2274 samples.Malaria was diagnosed in 239 samples by both QBC and smear.QBC allowed detection of an additional 89 cases.Among this 89 cases ,80 cases had low parasitemias and the conclusion offered was QBC is very useful in low parasitemia cases.

16. Urmila shenoi *et al*., 1996[62] , conducted a study in Department of Pathology in Kasturba Medical College with 18,845 population in which total number positive by QBC was 4,824.Thick film identified 3,490 cases. Out of the total number of 4,824 samples positive by QBC 4,795 were P. vivax species of malaria and 29 were P. falciparum species. All the 1334 samples which were

negative by the thick film and positive by QBC, had very low parasitemia, seen either in the platelet-plasma interface, or deep in the red cell layer. P. falciparum parasites were seen in both the QBC and the thick films in all the 29 cases. The QBC detected parasites when the thick film was negative in 1334 patients, indicating that QBC is more sensitive. Positive QBC tubes were never observed in non-infected individuals. Repeat thick films were studied in only 242, out of 1334 initially QBC positive and thick film negative samples. Only 189 out of the 242 samples tested positive by both the techniques, indicating that at least some of the "false positives" by the QBC were true positives with false negative blood films.

17. Ahmad et al.,2006[63] conducted a cross sectional study in Jinnah PG Medical Centre,Karachi in which he observed the incidence of P.falciparum was 44.4%.The major clinical features were pyrexia with or without chills and rigor, vomiting, pallor, hepatosplenomegaly.

18. Jadhav U M et al.,2004[64] with a sample size of 1565 cases of malaria analysed the thrombocytopenia in malaria and the correlation with type and severity with it. The mean platelet count

in P.vivax was 1.15 lakh/µl and in P.falciparum it was 1.00 lakh/µl. They concluded that absence of thrombocytopenia is uncommon in malaria and it's presence is not a distinguishing feature between the types.

19. Bhushan Katira and Ira Shah 2006[65], conducted a study on thrombocytopenia in P.vivax malaria with 5 children in Wadia Hospital, Mumbai. They concluded that thrombocytopenia in P.vivax malaria is increasing in India which usually disappears after treatment.

20. Alfonso J et al.,2005 [66] observed 78 children with P.vivax malaria over 3 year period and found that anemia was present in 94.87% with a mean haemoglobin concentration of 8 gm%. The mean platelet count was 1.27 lakhs.

STUDY JUSTIFICATION

The WHO released new guidelines (2010) for the treatment of malaria .This provides evidence based and current recommendations for the diagnosis of malaria and it's treatment. The newer guidelines emphasised the necessity for diagnosis before starting treatment. The main aim is to restrict the unnecessary use of antimalarials, so that the emergence and spread of drug resistance is reduced. Diagnostic tests should be done in all cases of suspected malaria including children < 5 years of age. With this revision, patients of all ages in all epidemiological settings with suspected malaria, should receive a parasitological confirmation of diagnosis by either microscopy or RDT. Treatment based on clinical diagnosis alone, should be reserved, where the facility for diagnostic tests are not available.

Though peripheral smear microscopy is considered as the gold standard method for diagnosing malaria, it is time consuming, labour intensive, requires considerable expertise for its interpretation. Even when performed by expert it has its own limitations and it shows variable sensitivity and specificity compared to the recent technical advances. Identification of P.vivax is no longer a problem by peripheral smear. As P.falciparum is sequestrated in organs, the level of circulating parasites

is low. So its identification is difficult. The inconsistent reliability of the smears has led to the practice advocated by some clinicians to simply employ a therapeutic trial of anti-malarial agents, out of utter desperation from the oftentimes reported negative malarial smear. In a country where malaria is endemic, a procedure which is sensitive enough to detect malarial parasite in a short span of time is a very much welcome diagnostic tool. This facilitates early diagnosis and thus timely therapy, averting the florid manifestations of malaria thus reducing morbidity and mortality.

To date RDTs & QBC have practically always been assessed against expert microscopy. It would be useful to determine whether the peripheral smear examination might not be replaced by some other more accurate standard, easy and quick diagnostic tests so that it can be applicable even in the peripheral settings where major facilities are not available. Worldwide many studies are available comparing RDT Vs PBS, QBC Vs PBS. In our state, no such study is available comparing QBC and RDT (pLDH) with peripheral smear especially in children.

AIM OF THE STUDY

Evaluating the diagnostic value of Quantitative Buffy Coat and Rapid Diagnostic Test (using pLDH) against the gold standard peripheral smear examination, in the diagnosis of malaria in children between 1month to 12 years of age group.

Secondary objective

To study the clinical profile of malaria cases detected by peripheral smear.

SUBJECTS & METHODS

1. Methodology

Study design : Descriptive study and case control study.

Study place : Medical wards of Institute of Child Health and Hospital for children, Egmore, Chennai.

Study period : January 2010 – December 2011

Study population

Inclusion Criteria :

Children between 1 month to 12 years of age group presented with fever more than 5 days with other clinical symptoms and signs of malaria like fever with chills and rigor, pallor, hepatosplenomegaly, etc..

Pallor : Palmar pallor is taken into account as per IMNCI guidelines.

Hepatomegaly : If the liver span for that particular age is more than the normal, it was taken as hepatomegaly.

Splenomegaly : palpable spleen is considered as splenomegaly.

Exclusion Criteria

Fever with obvious focus of infection like abscess, urinary tract infection (which will cause fever with chills) and children who had received treatment for malaria in the past 4 weeks.

Sample size: 150. Sample size was calculated based on observations from previous studies , as having sensitivity of 88% with 10% allowable error and 99% confidence.150 children satisfying the case definition and inclusion criteria are selected and subjected for peripheral smear, QBC & RDT (pLDH).

2. Manoeuvre

Children were enrolled on the basis of inclusion and exclusion criteria after obtaining parental consent. Using patient data entry form (annexure I) information was obtained regarding history and complaints. They were subjected for clinical examination and about three ml of blood sample was collected by venipuncture under aseptic precautions. The following investigations were carried out using the sample : QBC and RDT (pLDH) apart from routine investigations. Capillary blood was obtained by finger prick method for peripheral smear (thick and thin) examination.

Peripheral smear study

The smears were obtained as per the standard technique and stained with Leishman's stain. A minimum of 200 oil immersion fields (x 100 objective) were examined in the thick film. Following the detection of malarial parasites in a thick film, the thin film was examined to determine the species. If the malarial parasites were absent in the thick smear, the entire thin film was examined.

QBC

For QBC technique, approximately 60 µl of blood was taken into a capillary tube which is coated with acridine orange from black lined end and fitted with a cap. Then a plastic float was inserted inside the capillary tube and centrifugation was done. The tube was then mounted on a small plastic holder and examined by rotating the tube under ordinary light microscope with customized fluorescence.

RDT (pLDH)

Plasmodium lactate dehydrogenase immunochromatographic assay was done using the commercial kit DiaMed OptiMal-IT (flow,inc.,Portland,oreg).

The device was placed horizontally on a flat surface and patients name and date was written on the label. One drop of buffer to first (conjugate) well, and four drops to second (wash) well was added and waited for 1 minute. Blood was taken up to the black mark on the pipette. Entire volume of blood (10μml) was added to the first well. The mixture was gently stirred with the upper end of the pipette and allowed to stand for 1 minute, during which the lysis buffer disrupts the RBCs to release pLDH. The dipstick holder was pulled out, the legs of the dipstick holder was inserted into the holes besides the conjugate well, so that the dipstick end reaches the bottom of the conjugate well. For the next 8 min the blood/conjugate mixture is allowed to migrate to the top of the pLDH strip. The dipstick is transferred to the second well with the washing buffer which clears the haemoglobin from the strip. Once the reaction field is completely cleared of blood, and the control band is clearly visible, the dipstick was removed from the wash well and fixed back into the clear plastic frame & observed for the presence of any band and the corresponding letter C,P and Pf. Interpretation of the test result was done as below:

1. When one control band and two test bands (genus specific, species specific) appeared, the test was considered to be positive for P. falciparum.

2. When one control band and one test band appeared the test was considered positive for P. vivax.

3. When only control band appeared without test band the test was considered to be invalid.

4. Mixed infection with P.falciparum and other plasmodium species is identified by the presence of both genus specific, species specific bands. The genus specific band is much darker than the species specific band.

Peripheral smear, RDT, QBC were performed by 3 different persons for the sake of blinding. Investigation results were tabulated and analysed.

3. Statistical analysis

Data was entered in microsoft office excel sheet and statistical operations were done through SPSS for windows version 17.All the univariate analysis were done by chi square test. For continuous measurements two sample t test was done. Sensitivity, specificity, positive predictive value, negative predictive value, positive and negtive liklihood ratios and diagnostic accuracy were calculated for QBC and RDT(pLDH) by comparing the results with the gold standard peripheral smear study.

4. Ethics

Written informed consent was obtained from the parents and Institution review board clearance was obtained.

OBSERVATIONS

Peripheral smear positive cases	:	66
P.vivax	:	60 cases
P.falciparum	:	6 cases
RDT (pLDH) positive cases	:	82
P.vivax	:	74 cases
P.falciparum	:	8 cases
QBC positive cases	:	86
P.vivax	:	78 cases
P.falciparum	:	8 cases.

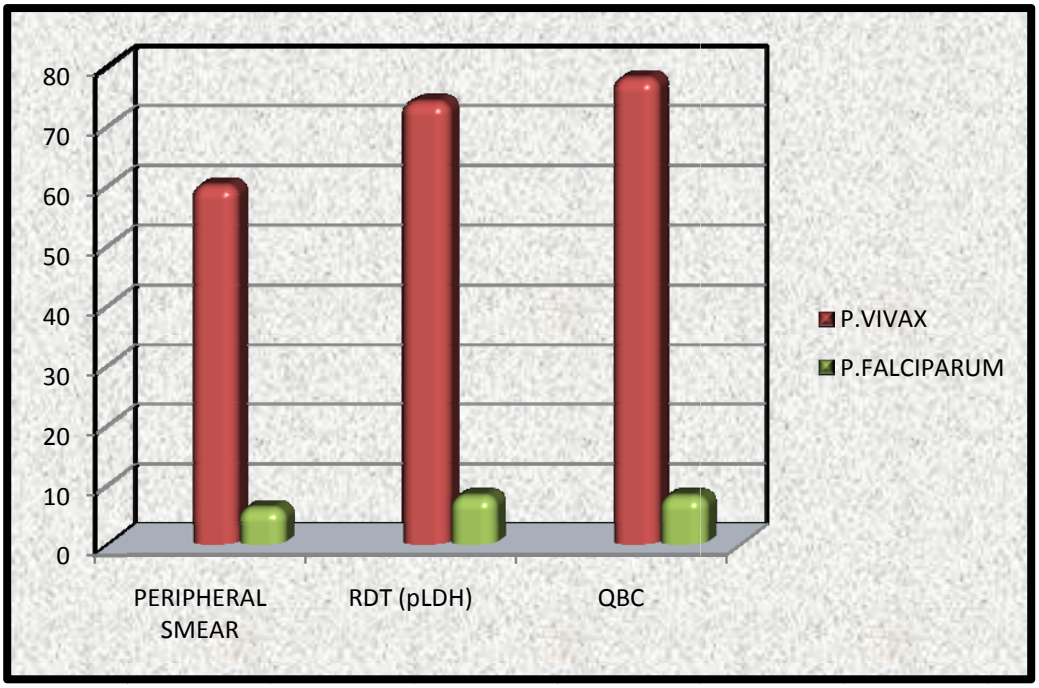
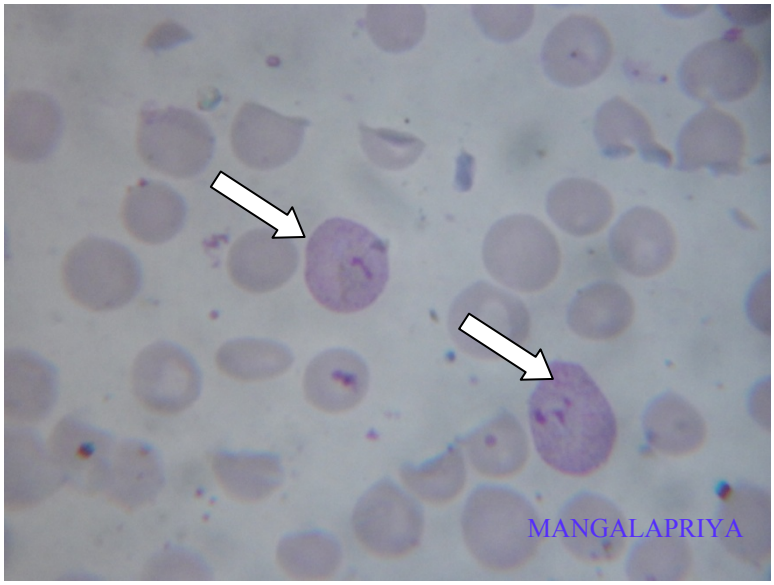


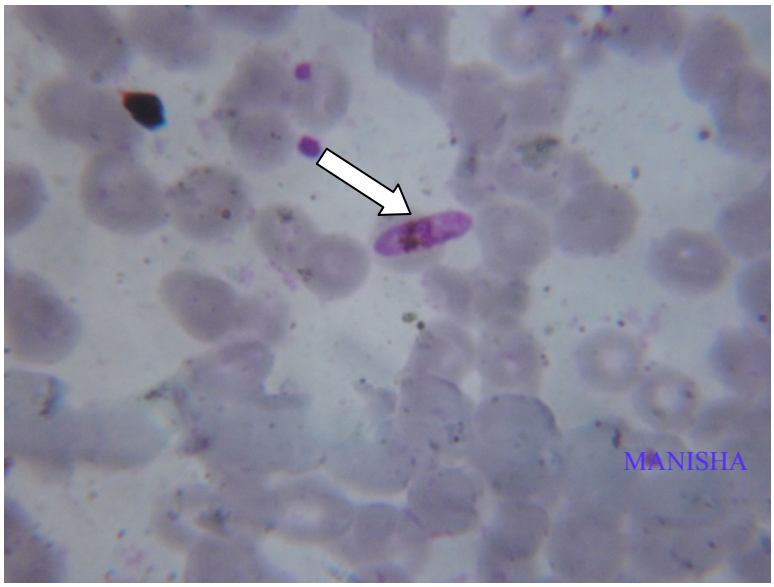
Table 4: Comparison of QBC, RDT (pLDH) with peripheral smear positive results-species wise

Tests	Results	P.vivax N(%)	P.falciparum N(%)	Total (N=150)
Peripheral smear	Positive	60(90.9%)	6(9.1%)	66 (44%)
QBC	Positive	78(90.7%)	8(9.3%)	86(57.3%
	Negative	-	-	
RDT	Positive	74(90.2%)	8(9.8%)	82(54.7%)
	Negative	-	-	

PERIPHERAL SMEAR EXAMINATION

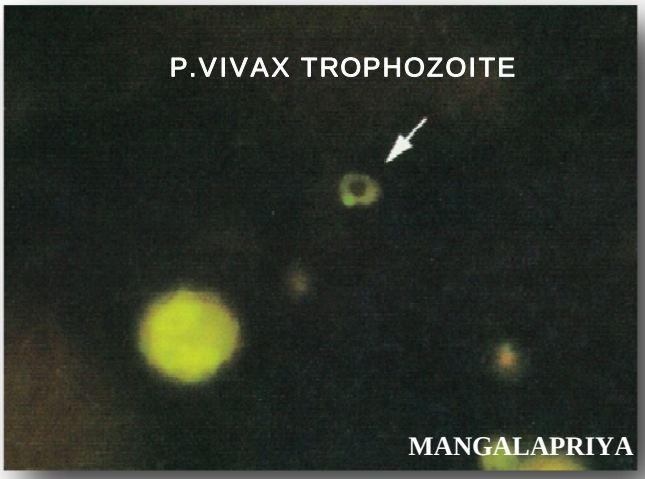


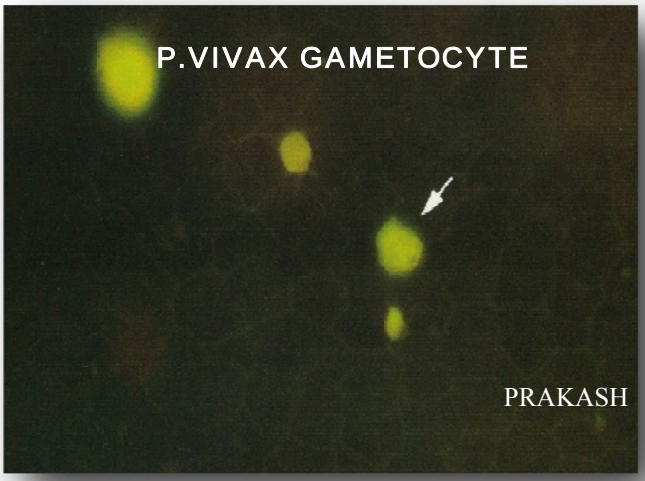
PLASMODIUM VIVAX - TROPHOZOITES

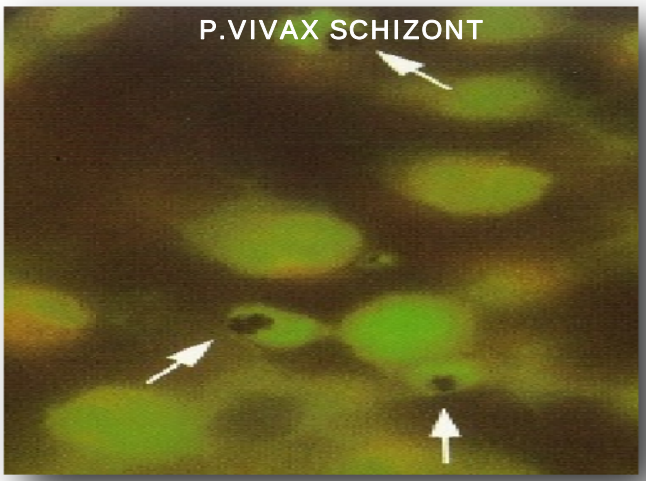


PLASMODIUM FALCIPARUM GAMETOCYTE

QUANTITATIVE BUFFY COAT TECHNIQUE: P.VIVAX

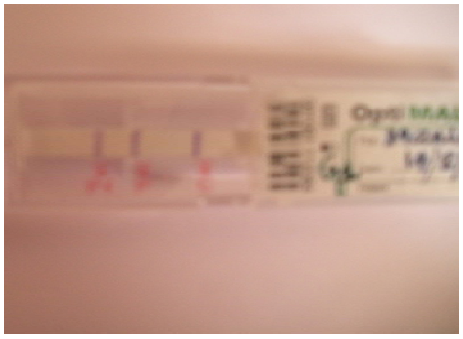




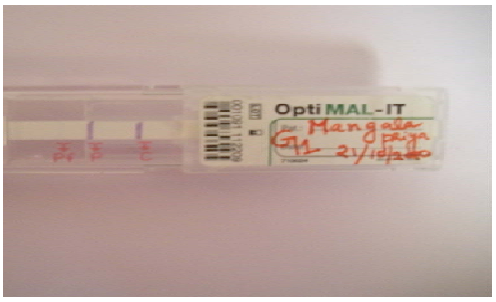


QUANTITATIVE BUFFY COAT TECHNIQUE: P.FALCIPARUM

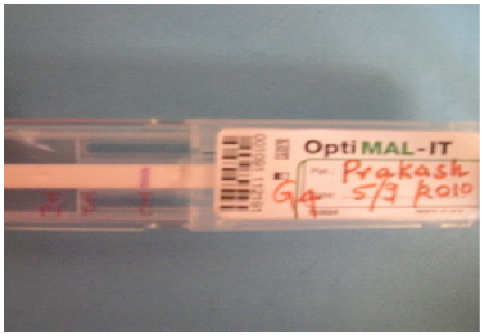
RAPID DIAGNOSTIC TEST (PLASMODIUM LACTATE DEHYDROGENASE)



P.falciparum



P.vivax



RDT Negative for Malaria

Table 5 : Correlation of clinical features and investigations with peripheral smear results - univariate analysis

Variables		Peripheral smear positive	Peripheral smear negative	OR	95% C.I	P value
Myalgia	Present	10(15.2%)	23.8%	0.188	0.2,1.3	0.571
	Absent	56(84.8%)	76.2%			
Chills	Present	13(19.7%)	26.2%	0.351	0.3,1.5	0.691
	Absent	53(80.3%)	73.8%			
GIT symptoms	Present	27(40.9%)	61.9%	0.426	0.2,0.8	0.11
	Absent	39(59.1%)	38.1%			
Headache	Present	11(16.7%)	8.3%	2.2	0.8,6	0.12
	Absent	55(83.3%)	91.7%			
Seizure	Present	3(8.3%)	8.3%	0.524	0.1,2.1	0.36
	Absent	63(91.7%)	91.7%			
Altered sensorium	Present	O(0%)	4.8%	0.548	0.,0.6	0.07
	Absent	66(100%)	95.2%			
Renal symptoms	Present	1(1.5%)	1.5%	0.415	0.04,4	0.44
	Absent	65(98.5%)	98.5%			

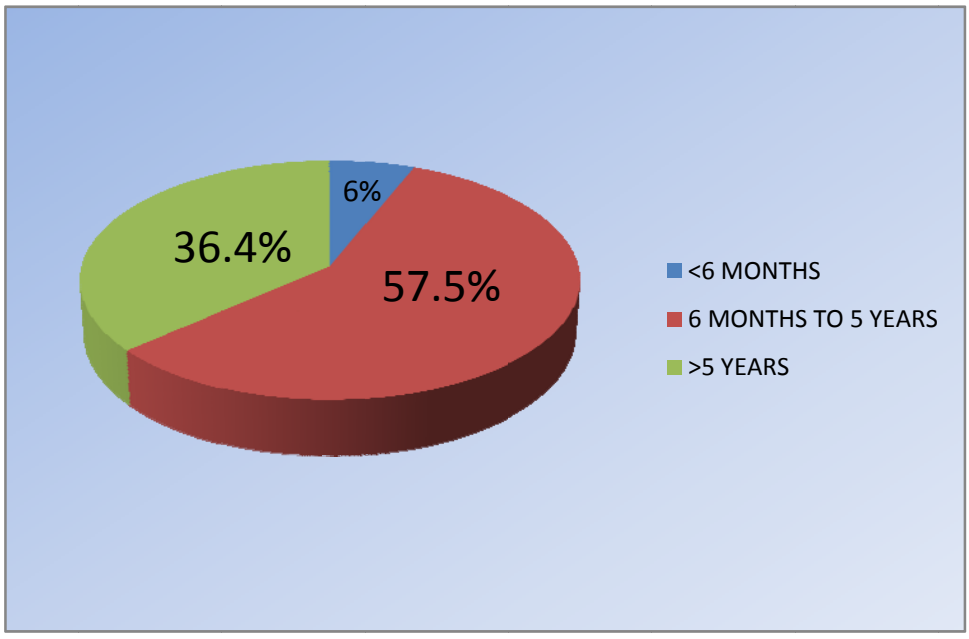
Pallor	Present	36(54.5%)	40.5%	1.765	0.9,3.3	0.09
	Absent	30(45.5%)	59.5%			
Icterus	Present	3(4.5%)	0%	0.429	0.3,0.5	0.05
	Absent	63(95.5%)	100%			
Edema	Present	0(0%)	2.4%	0.558	0.48,0.6	0.21
	Absent	66(100%)	97.6%			
Hepatomegaly	Present	8(12.1%)	15.5%	0.75	0.29,1.9	0.56
	Absent	58(87.9%)	84.5%			
Splenomegaly	Present	14(21.2%)	17.9%	1.238	0.5,2.7	0.60
	Absent	52(78.8%)	82.15			
Hepato Splenomegaly	Present	36(54.55%)	41.7%	1.680	0.87,3.2	0.12
	Absent	30(45.5%)	58.3%			
Anemia	Present	44(66.6%)	17.8%	9.2	4.3,19.6	0.000
	Absent	22(33.4%)	82.2%			
Thrombo - cytopenia	Present	38(57.5%)	2%	55.64	12.6,245	0.000
	Absent	28(42.5%)	98%			

From table 5, we can infer that no individual clinical parameter is statistically significant with peripheral smear results, which necessitates the need for diagnostic tests for the diagnosis of malaria.

Anemia was detected among 44 cases of smear positive malaria.The possibility of a child having anemia is 9.2 times more common among smear positive cases when compared to smear negative cases Odds Ratio (95% confidence interval)=9.2(4.3-19.6).

Thrombocytopenia was observed in 38(57.5%) of malaria cases with the Odds Ratio of 55.64 and 95% confidence interval of 12.6-245 which was statistically very much significant.The other investigations (continuous variables) like total count, blood sugar and urea,serum creatinine and bilirubin were not statistically significant when analysed with two sample t test between smear positive and smear negative cases.

Age distribution: About 57.5 % of the malaria cases were distributed in the 6 months to 5 years age group.

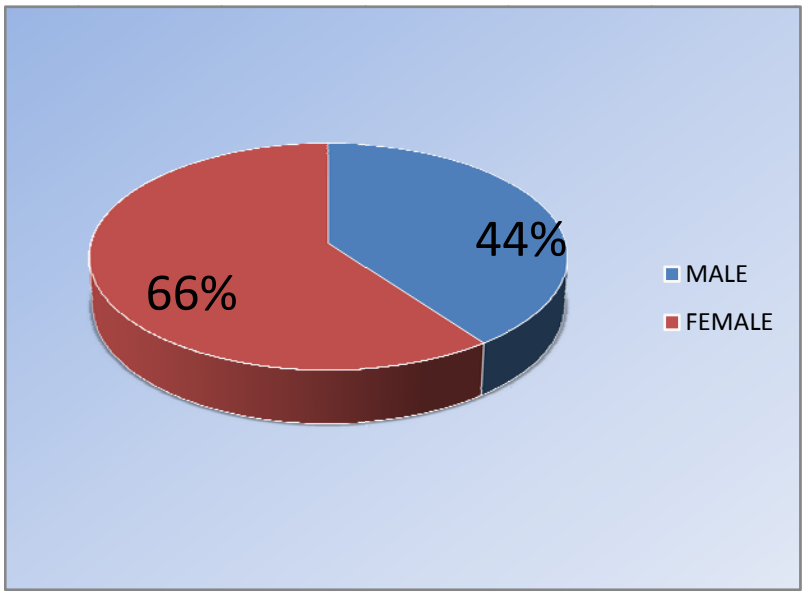


<6 months - 4(6%)

6 months-5years - 38(57.5%)

>5 years - 24(36.4%)

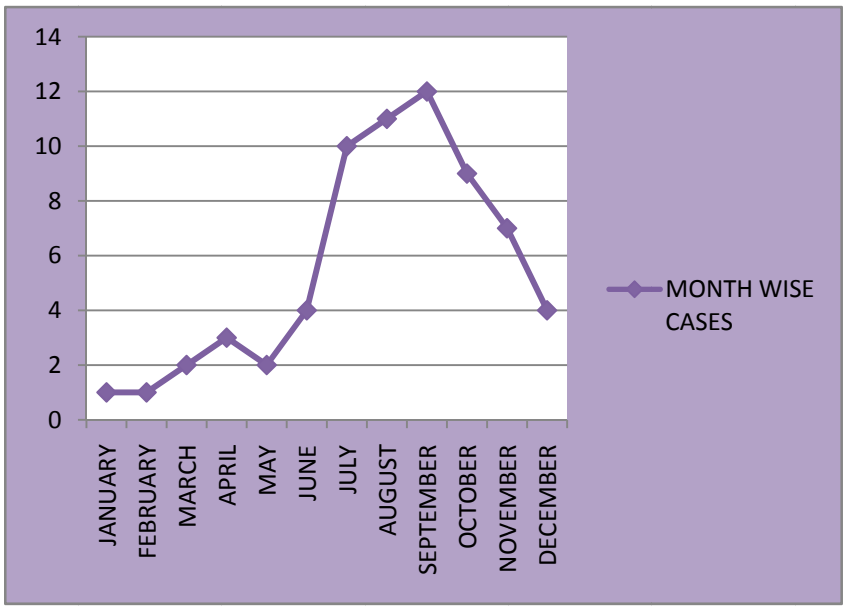
Gender distribution



Total smear positive cases:66

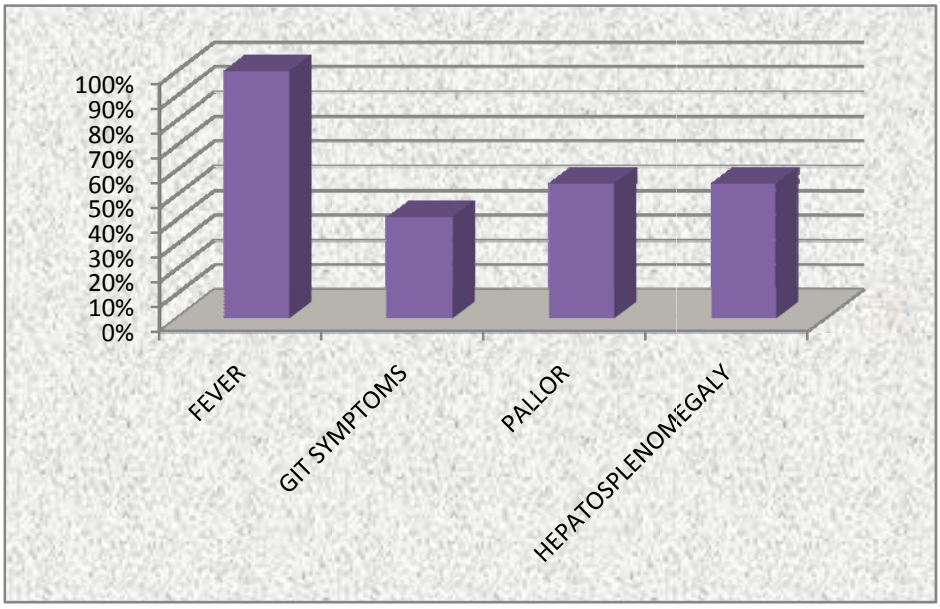
Males - 29(44%)
Females - 37(66%)

Trend in the incidence of malaria cases during the StudyPeriod



Maximum number of cases were observed between July to November.

Major clinical features of malaria



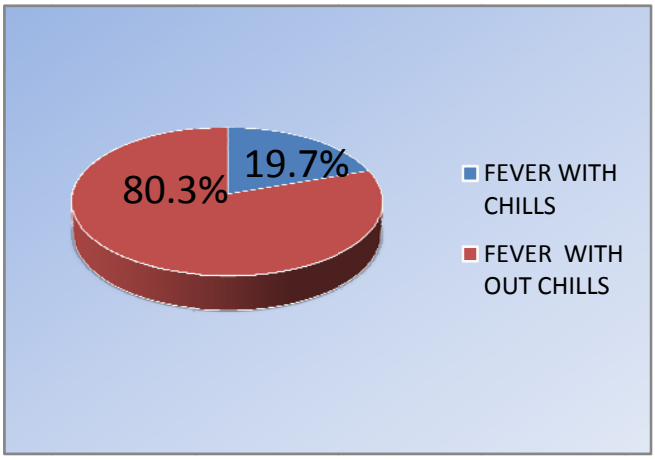
Fever : 100%

GIT symptoms:27(40.9%)

Pallor:36(54.5%)

Hepatosplenomegaly:36(54.5%)

Fever

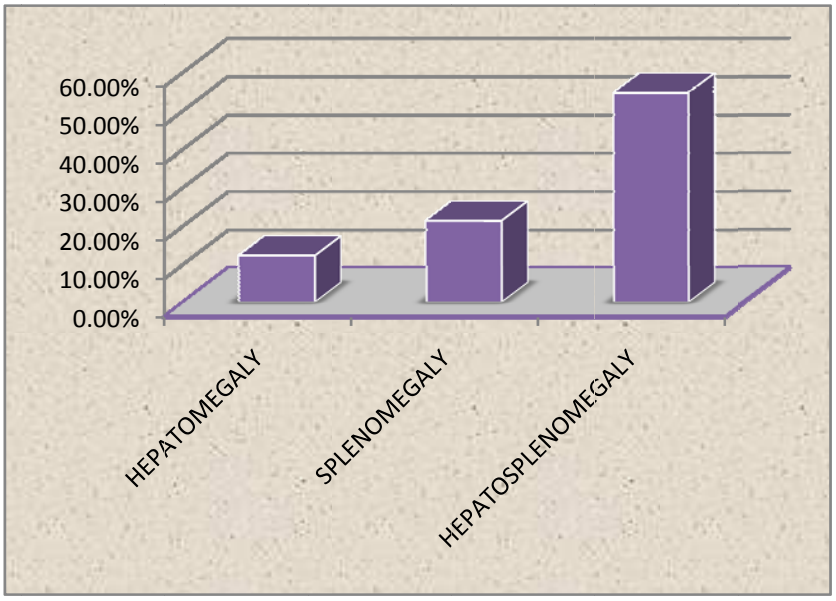


The mean for fever duration was 8.5 days (SD 5.07)

Feverwithout chills : 53 (80.3%)

Fever with chills : 13 (19.7%)

Organomegaly in malaria

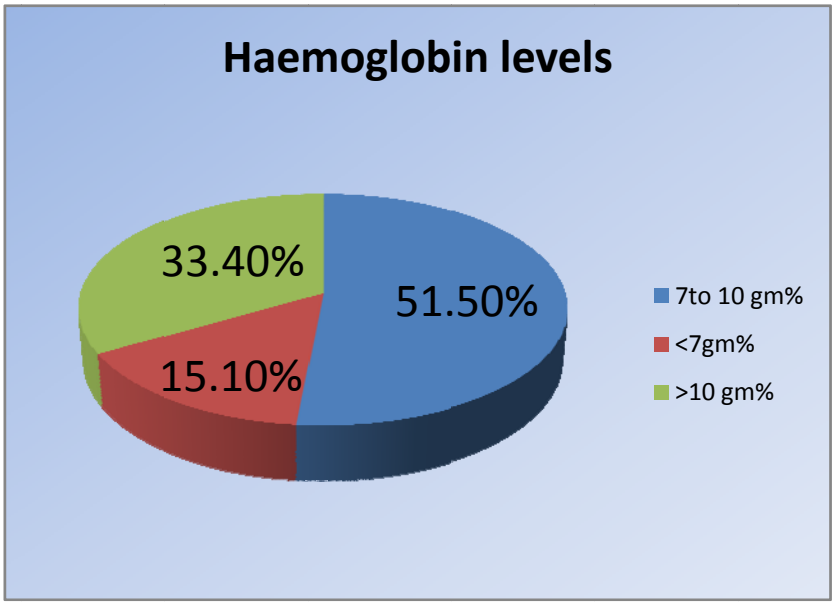


Hepatomegaly : 8(15.5%)

Splenomegaly : 14(21.2%)

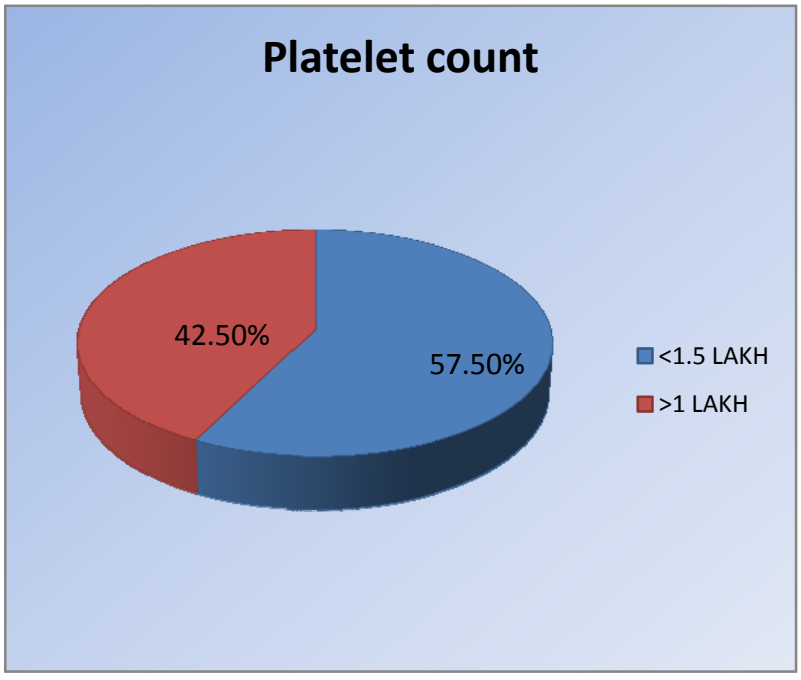
Hepatosplenomegaly : 36(54.5%)

Laboratory investigations



Patients with anemia(< 10 gm%):44(66.6%)

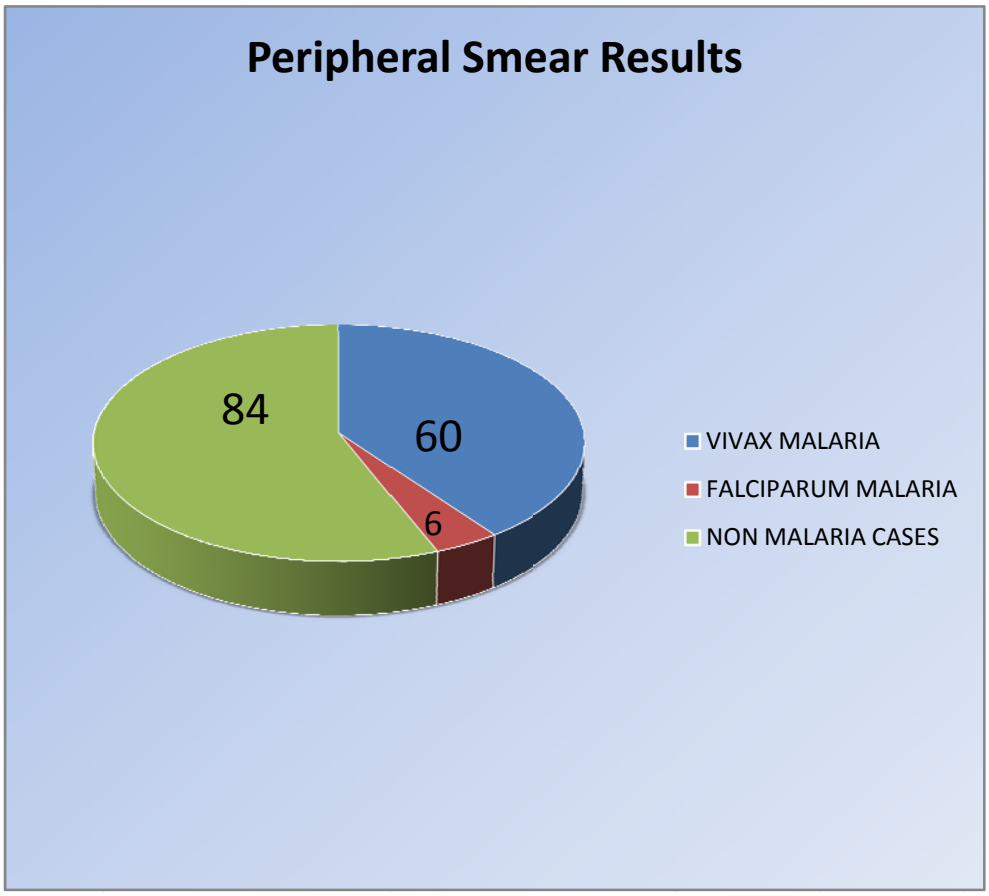
Patients without anemia(>10gm%):22(33.4%)



Patients with platelet count <1.5 lakh: 38 (57.5%)

Patients with platelet count >1.5 lakh: 28(42.5%)

Diagnostic evaluation of QBC,RDT (pLDH) in comparison with peripheral smear study



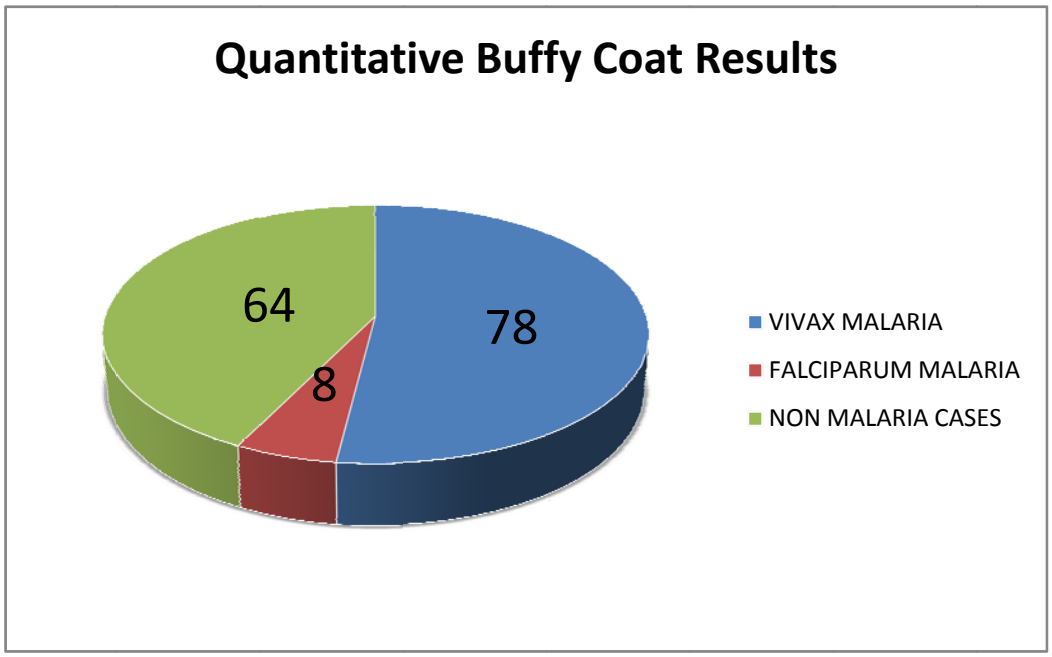


Table 6: Peripheral Smear Vs QBC (Vivax & Falciparum)

Tests	Results	Peripheral Smear (Vivax &Falciparum)		
		Positive	Negative	Total
QBC(Vivax &Falciparum)	Positive	66	20	86
	Negative	0	64	64
	Total	66	84	150

Results of Peripheral smear Vs QBC (P.vivax & P.falciparum)

	Estimate	95% Confidence Interval
Sensitivity	100%	94.5- 100
Specificity	76.19%	66.06-84.03
Positive predictive value	76.74%	69.79-84.41
Negative predictive value	100%	94.34-100
Diagnostic accuracy	86.67%	80.3-91.2
Liklihood ratio of positive test	4.2	3.81-4.63
Liklihood ratio of negativetest	0.0	0.0

Table 7: Peripheral Smear Vs QBC (Vivax)

Tests	Results	Peripheralsmear(Vivax)		
		Positive	Negative	Total
QBC(Vivax)	Positive	60	18	78
	Negative	0	72	72
	Total	60	90	150

Results of Peripheral smear Vs QBC (P.vivax)

	Estimate	95% Confidence Interval
Sensitivity	100%	93.98- 100
Specificity	80%	70.59- 86.96
Positive Predictive Value	76.92%	66.44-84.87
Negative Predictive Value	100%	94.93-100
Diagnostic Accuracy	88%	81.83-92.27
Liklihood Ratio of Positive Test	5	4.484-5.575
Liklihood Ratio of Negativetest	0.0	0.00

Table 8: Peripheral Smear Vs QBC (Falciparum)

		Peripheralsmear (Falciparum)		
		Positive	**Negative**	**Total**
QBC (Falciparum)	**Positive**	6	2	8
	Negative	0	142	142
	Total	6	144	150

Results of Peripheral smear Vs QBC (P.falciparum)

	Estimate	95% Confidence Interval
Sensitivity	100%	60.97-100
Specificity	98.61%	95.08- 99.62
Positive predictive value	75%	40.93-92.85
Negative predictive value	100%	97.37-100
Diagnostic accuracy	98.67%	95.27-99.63
Liklihood ratio of positive test	72	27-191.8
Liklihood ratio of negativetest	0.0	0.0

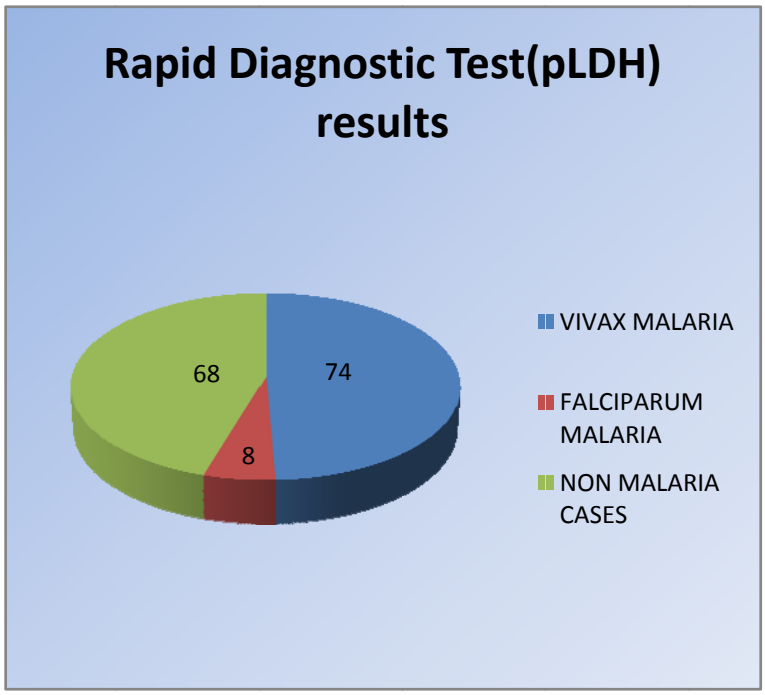


Table 9: Peripheral Smear Vs RDT (pLDH) – Vivax & Falciparum

Tests	Results	Peripheral Smear (Vivax&Falciparum)		
		Positive	Negative	Total
RDT	Positive	65	17	82
	Negative	1	67	68
	Total	66	84	150

Results of Peripheral smear Vs RDT (P.vivax & P.falciparum)

	Estimate	95% Confidence Interval
Sensitivity	98.48%	91.9- 99.73
Specificity	79.76%	69.96- 86.96
Positive predictive value	79.27%	69.28-86.63
Negative predictive value	98.53%	92.13-99.74
Diagnostic accuracy	88%	81.83-92.27
Liklihood ratio of positive test	4.86	4.33-5.46
Liklihood ratio of negativetest	0.019	0.002-0.136

Table 10: Peripheral Smear Vs RDT (pLDH) – Vivax

		Peripheralsmear(Vivax)		
		Positive	**Negative**	**Total**
RDT(Vivax)	**Positive**	59	15	74
	Negative	1	75	76
	Total	60	90	150

Results of Peripheral smear Vs RDT (pLDH)-Vivax

	Estimate	**95% Confidence Interval**
Sensitivity	98.33%	91.14- 99.71
Specificity	83.33%	74.31- 89.63
Positive predictive value	79.73%	69.21-87.31
Negative predictive value	98.68%	92.92-99.77
Diagnostic accuracy	89.33%	83.38-93.33
Liklihood ratio of positive test	5.9	5.17-6.727
Liklihood ratio of negativetest	0.02	0.002-0.142

Table 11 : Peripheral Smear Vs RDT (pLDH) – Falciparum

		Peripheralsmear(Falciparum)		
		Positive	**Negative**	**Total**
RDT (Falciparum)	**Positive**	6	2	8
	Negative	0	142	142
	Total	6	144	150

Results of Peripheral smear Vs RDT(pLDH)- P.falciparum

	Estimate	**95% Confidence Interval**
Sensitivity	100%	60.7-100
Specificity	98.61%	95.08- 99.62
Positive predictive value	75%	40.93-92.85
Negative predictive value	100%	97.37-100
Diagnostic accuracy	98.67%	95.27-99.63
Liklihood ratio of positive test	72	27.02-191.8
Liklihood ratio of negativetest	0.00	0.00

Table 12: Comparison of diagnostic value of QBC and RDT (pLDH) against peripheral smear for the diagnosis of malaria

Tests		Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy	Likelihood ratio of positive test	Likelihood ratio of negative test
QBC	P.vivax & P.falciparum	100%	76.19%	76.74%	100%	86.67%	4.2	0.00
	P.Vivax	100%	80%	76.92%	100%	88%	5	0.00
	P.falciparum	100%	98.61%	75%	100%	98.67%	72	0.00
RDT	P.vivax & P.falciparum	98.48%	79.76%	79.27%	98.53%	88%	4.86	0.019
	P.vivax	98.33%	83.33%	79.73%	98.68%	89.33%	5.9	0.02
	P.falciparum	100%	98.61%	75%	100%	98.67%	72	0.00

DISCUSSION

In our study,clinical suspicion of malaria was made in 150 children.Among them only 66 were diagnosed to have malaria on the basis of smear positivity. As the symptoms and the signs of malaria are vague, the clinical diagnosis of malaria is very difficult and some times the over enthusiastic diagnosis made on clinical grounds, lead on to unnecessary use of antimalarials, the major cause of emerging drug resistance. So we have to depend on the Laboratory investigations for accurate diagnosis.

Age group:

Majority of cases of malaria in our study were between 6 months to 5 years of age group, which coincides with the study of Ahmad et al.[63]

Gender

Female to male ratio is 1.2:1

Seasonal distribution :

The maximum number of cases (38) were observed during July to October with the peak incidence noted in the month of September, which coincides with the monsoon and post monsoon

period. This was similar to the earlier observations by Ahmad et al.[63]

Clinical features:

Fever was present in all (100%) cases of confirmed malaria on the day of admission.The characteristic fever with chills and rigor was observed in 19.7% of patients only.Next to fever,the commonest complaint is GIT symptoms (40.9%) in the form of nausea,vomiting,anorexia,abdominal pain and loose stools.16.7% of cases had headache and 15.2% had muscle ache. Two (8.3%) patients had seizures.Both were turned out to have falciparum malaria.

On examination, pallor was observed clinically in 36 (54.5%) cases.Icterus was noted in 3 (4.5%) cases.This is due to haemolysis as evidenced by low haemoglobin levels in them. 8 (12.1%) patients had hepatomegaly, 14 (21.2%) had splenomegaly, 36 (54.5%) had hepatosplenomegaly.

The majority of children with malaria in our study, had GIT symptoms, pallor and hepatosplenomegaly in addition to fever which was similar to the earlier observation by Ahmad et al.[63]

Investigations

Among the malaria cases, 66.6% of patients had haemoglobin levels of 7 -10 gm% and 15.1% of cases had <7 gm%.In our study,the incidence of anemia in malaria detected by peripheral smear is more when compared to the study by Narayanappa et,al[45] whose predominant study population with malaria was between 9-12 years of age. The increased incidence of anemia in our series may be explained by the superadded nutritional anemia observed in younger children.

Among the malaria cases detected by smear, 57.5% had platelet count of <1.5 lakh which is similar to the study observed by Jadhav *et al*[64]

Evaluation of tests

Time requirement

Peripheral smear:60 to 90 minutes

QBC: Parasites can be detected within few minutes in a positive sample. About 10 minutes are required for a negative sample. (totally 15 minutes including centrifugation).

RDT(pLDH) : 10 minutes.

Of the 150 cases, 66(44%)cases were positive for malaria and 84(56%) cases were negative for malaria by peripheral smear microscopy. Out of 66 malaria cases, 60 (90.1%) were positive for P.vivax and 6 were positive for P.falciparum(9.9%) . QBC test was positive in 86 cases and RDT(pLDH) was positive in 82 cases.The sensitivity,specificity,positive predictive value,negative predictive value, positive liklihood ratio ,negative likelihood ratio and diagnostic accuracy of QBC and RDT for P.vivax, P.falciparum was compared with peripheral smear. (Table 6 to 11)

QBC analysis:

The QBC detected 86 cases of malaria of which, 78 (90.7%)were positive for P.vivax and 8 (9.3%) were positive for P.falciparum.The cases which were positive by smear, were also detected by the QBC method. In addition twenty cases which were not detected by smear, were diagnosed as malaria by QBC technique which includes two P.falciparum cases also. All patients who were malaria parasite negative by QBC method were also smear negative.

The QBC method showed sensitivity,specificity,positive predictive value and negative predictive value of 100%, 80%, 76.92%, 100% for P.vivax and 100%, 98.61%, 75%, 100% for P.falciparum . The positive likelihood ratios for detection of P.vivax is 5 and for falciparum it is 72.The negative likelihood ratio gfor detection of P.vivax is 0 and for P.falciparum also it is 0.0. Similar to earlier observations by Pinto et al.,[61] Krishna B V and Deshpande et al.,[19] and Urmila shenoi et al[63]., our study identified 20 cases of probable malaria (including two P.falciparum cases) which were negative by peripheral smear. These patients were treated with antimalarials and showed good clinical improvement. The reason for smear negativity may be because of low parasitemia as observed by srinivasan et al.,[16] and palmer et al.,[48]

Rapid diagnostic test analysis (OptiMAL-IT using pLDH):

The RDT(pLDH) test showed 82 cases were positive for malaria.Among the 82 cases, 74 (90.2%) were positive for P.vivax and 8 were positive for P.falciparum (9.8%). A case which was negative by RDT (pLDH) test was found to be positive by smear as well as QBC. This could be attributed to low antigen levels as observed by Iqbal et al[28]. This test identified 17 additional cases of

malaria which were negative by peripheral smear.All these 17 cases were also positive by QBC and showed good response to antimalarials.

Two cases of P.falciparum were missed by peripheral smear method, found to be positive by both both QBC and RDT methods.No mixed infection was identified by any of these methods.No mortality has been observed in our study.

The RDT method showed sensitivity,specificity,positive predictive value and negative predictive value of 98.33%, 83.33%, 79.73%, 98.68% for P.vivax and 100%, 98.61%, 75%, 100% for P.falciparum . The sensitivity rate is comparable to study of Iqbal et al[51]and quitana et al [49] .The positive likelihood ratios for detection of P.vivax is 5.9 and for P.falciparum it is 72. The negative likelihood ratios for detection of p.vivax is 0.02 & for falciparum also it is 0.0.

SUMMARY

1. Fever with chills,the classical feature of malaria was present in only 19.1% of malaria cases detected by peripheral smear.Next to fever,GIT symptoms were predominant.
2. Among the malaria cases detected by peripheral smear 66.6% had anemia and 57.5% had thrombocytopenia.
3. Peripheral (thick and thin) blood smear examination is considered to be the gold standard for the diagnosis of malaria.In setups where the technicians are overloaded with hundreds of samples per day, the chance of missing the smear with a low parasite count is more.
4. QBC is a much simpler, rapid and highly sensitive diagnostic test to detect both vivax and falciparum malaria. The only disadvantage of QBC is, requirement of specialized instrumentation.
5. RDT (pLDH) is highly sensitive in picking up falciparum cases, which is equal to that of QBC. So it can represent a diagnostic tool for falciparum malaria where expert microscopy/QBC is not available. The sensitivity of RDT(pLDH) in diagnosing vivax malaria is much lower than the peripheral smear microscopy and QBC.

6. The technique and interpretation of RDT(pLDH) is much easier when compared to peripheral smear and QBC. RDT (pLDH) can be useful in areas where specialized laboratories or microscopy are unavailable and when urgent malaria diagnosis is needed by a practitioner without the delay associated with the laboratory diagnosis.

CONCLUSION

In future the peripheral smear examination can be replaced by Quantitative Buffy Coat technique for the diagnosis of malaria.

In resource limited settings where specialized instrumentation facilities are not available, RDT (pLDH) can be used to diagnose malaria.

LIMITATION

In our study, the laboratory technician is utilized for doing other investigations like haemoglobin, blood counts, etc., in addition to peripheral smear examination. Due to the workload, the time spent on each smear by the technician may be less. This may be a reason for low detection rates of malaria by peripheral smear. If a dedicated person is allotted for doing peripheral smear examination only, the performance would be better.

BIBLIIOGRAPHY

1. World malaria report 2010 : page 25. Available from http://www.who.int/malaria/world_malaria_report_2010/en/index.html.

2. Ashok S Kapse . Malaria in children. IAP text book of paediatrics 2009;4:423.

3. Brooks MI, Singh N, Hamer DH. Control measures for malaria in pregnancy in India. Indian Journal of Medical Research. Sep 2008 Available from http://findarticles.com/p/articles/mi_qa3867/is_3_128/ai_n32060546

4. Malaria disease burden-Directorate of Public Health and Preventive Medicine. Available from http://www.tnhealth.org/dphfacts/malaria.htm.

5. Barnish G, Bates I, Iboro J. Newer drug combinations for malaria.BMJ 2004;328: 1511–1512.

6. Malaria biology-CDC division of vector Borne Infectious Diseases. Available from http://www.cdc.gov/malaria/about/biology/index.html.

7. Ashok S Kapse. Malaria in children.IAP text book of paediatrics 2009;4:426-427.

8. Castelli F, Carosi G. Diagnosis of malaria infection .Handbook of malaria infection in the tropics.1997;15: 114.

9. Warhurst DC, William J. Laboratory diagnosis of malaria ACP Broadsheet No:148 J, Clinical Pathology1996;49:533-8.

10. Long GW, Jones TR, Rickman LS, Fries L, Egan J, Wellde B *et al*. Acridine orange diagnosis of plasmodium falciparum; evaluation after Experimental infection. The American Society of Tropical Medicine and Hygiene 1994; 51(5): 613-16.

11. Payne D. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. Bull World Health Organisation. 1988;66:621-628.

12. Anonymous. The laboratory diagnosis of malaria. The Malaria Working Party of The General Haematology Task Force of the

British Committee for Standards in Haematology. Clin Lab Haematol1997; 19*:* 165–170.

13. *Trape JF. Rapid evaluation of malaria parasite density and standardization of thick smear examination for epidemiological investigations.* Trans R Soc Trop Med Hyg 1985;79*:*181–*184.*

14. *World Health Organization,* 1991. *Basic Malaria Microscopy. Geneva: WHO.*

15. Fleischer B. Editorial: 100 years ago: Giemsa's solution for staining of plasmodia. Trop Med Int Health2004; 9: 755–756.

16. Srinavasan S, Moody A H , Chiodini P L. Comparison of blood-film microscopy, the optimal® dipstick, Rhodamine 123 and PCR for monitoring anti-malarial treatment. Ann. Trop. Med. Parasitol 2000; 94:227–232

17. Cooke , Morris-Jones S, Horton J, Greenwood B M, Moody A H, Chiodini P L. *Evaluation of benzothiocarboxypurine for malaria*

diagnosis in an endemic area. Trans. R. Soc. Trop. Med. Hyg1993; 87:549

18. Marieb, Elaine N. Human Anatomy & Physiology 2007; ISBN 0-8053- 5910-9.

19. Krishna B V, Deshpande A R. Comparison between conventional and QBC methods for diagnosis of malaria. Indian J Pathol Microbiol 2003; 46:517-20

20. *Laferl H, Kandel K, Pichler H .False positive dipstick test for malaria.* N Engl J Med 1997; 337*:* 1635–*1636.*

21. *Histidine rich protein 2-malaria.farch.net. Available at http//www.meduniwien.ac.at/u*ser/herald.noedl/malaria/hrp2.html

22. *Mishra B, Samantaray J C, Kumar A, Mirdha B R. Study of false positivity of two rapid antigen detection tests for diagnosis of plasmodium falciparum malaria.* J Clin Microbiol 1999; 37*:* 1233.

23. *Laferl H, Kandel K, Pichler H. False positive dipstick test for malaria.* N Engl J Med 1997; 337*:* 1635–*1636.*

24. *Grobusch M P, Alpermann U, Schwenke S, Jelinek T, Warhurst D C. False-positive rapid tests for malaria in patients with rheumatoid factor.* Lancet1999; 353*:* 297-99.

25. *Mishra B, Samantaray J C, Kumar A, Mirdha B R. Study of false positivity of two rapid antigen detection tests for diagnosis of plasmodium falciparum malaria.* J Clin Microbiol 1999; 37*:* 1233.

26. Makler , M. T , Ries J M , Williams J A, Bancroft J E, Piper R C, Gibbins B L, and Hinrichs D J. Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. Am. J. Trop. Med. Hyg 1993;48:739–741

27. Piper, R., Lebras, J., Wentworth, L., Hunt-Cooke, A., Houze, S.,Chiodini, P. & Makler, M. Immunocapture diagnostic assays for malaria using Plasmodium lactate dehydrogenase (pLDH). American Journal of Tropical Medicine and Hygiene1999; 60:109-118.

28. Iqbal, J., P. R. Hira, A. Sher, and A. A. Al-Enezi. Diagnosis of imported malaria by Plasmodium lactate dehydrogenase and

histidine-rich protein 2 based immunocapture assays. Am. J. Trop. Med. Hyg 2001; 64:20–23

29. *Bruce-Chwatt LJ. DNA probes for malaria diagnosis. Lancet 1984;1:795*

30. Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown K N.Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol Biochem Parasitology 1993; 58: 283–292.

31. *Levine R A, Wardlaw S C, Patton C L. Detection of haematoparasites using quantitative buffy coat analysis tubes.* Parasitol Today1989; 5*:* 132*–134Chotivanich*

32. *Chotivanich K, Silamut K, Day, N P J. Laboratory diagnosis of malaria infection – a short review of methods. Aust J Med Sci 2006; 27:11-15.*

33. Makler M T, Palmer C J, Ager A L. A review of practical techniques for the diagnosis of malaria. Ann Trop Med Parasitology1998;92:419–433.

34. Rakotonirina H, Barnadas C, Raherijafy R, Andrianantenaina H, Ratsimbasoa A, Randrianasolo L, Jahevitra M, Andriantsoanirina V, Ménard D. Accuracy and reliability of malaria diagnostic techniques for guiding febrile outpatient treatment in malaria-endemic countries. Am J Trop Med Hyg 2008;78:217–221.

35. Morassin B, Fabre R, Berry A, Mangnaval JF. One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. Am J Trop Med Hyg 2002; 66:503-508.

36. Swan H, Sloan L, Muyombwe A, Chavalitshewinkoon-Petmitr P, Krudsood S, Leowattana W, Wilairatana P, Looareesuwan S, Rosenblatt J. Evaluation of a real-time polymerase chain reaction assay for the diagnosis of malaria in patients from Thailand. Am J Trop Med Hyg 2005;73:850–854.

37. Hawkes M, Kain KC. Advance in malaria diagnosis. Expert Rev Anti Infect Ther. 2007;5:1–11.

38. Poon L L, Wong B W, Ma E H, Chan K H, Chow L M, Abeyewickreme W, Tangpukdee N, Yuen KY, Guan Y,

Looareesuwan S, Peiris JS. Sensitive and inexpensive molecular test for falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. Clin Chem 2006;52:303–306.

39. Han ET, Watanabe R, Sattabongkot J, Khuntirat B, Sirichaisinthop J, Iriko H, Jin L, Takeo S, Tsuboi T. Detection of four *Plasmodium* species by genus- and species-specific loop-mediated isothermal amplification for clinical diagnosis. J Clin Microbiol 2007;45:2521–2528.

40. Aonuma H, Suzuki M, Iseki H, Perera N, Nelson B, Igarashi I, Yagi T, Kanuka H, Fukumoto S. Rapid identification of *Plasmodium*-carrying mosquitoes using loop-mediated isothermal amplification. Biochem Biophys Res Commun. 2008;376:671–676

41. *Hanscheid T. Diagnosis of malaria: a review of alternatives to conventional microscopy.* Clin Lab Haematology 1999;21*:* 235–*245*.

42. *Levine R A, Wardlaw S C, Patton C L. Detection of haematoparasites using quantitative buffy coat analysis tubes.* Parasitol Today1989; 5*:* 132*–134.*

43. *Kawamoto F. Rapid diagnosis of malaria by fluorescence microscopy with light microscope and interference filter.* Lancet 1991;337*:*200*–202.*

44. Parija S C, Rahul Dhodapkar, Subashini Elangovan, Chaya D R. A comparative study of blood smear, QBC and antigen detection for diagnosis of malaria.indian journal of pathology & microbiology2009; 52 (2):200-202.

45. Narayanappa D,Rajani H S,Jagadishkumar K,Keerthi B J.Rapid Diagnosis of Malaria Using Plamodium Lactate Dehydrogenase (OptiMAL)test. pediatric infectious diseases 2009Jan-Mar; vol 1:2-6.

46. Manjunath P. Salmani . Preeti B. Mindolli ,Basavaraj V. Peerapur Comparative Study of Peripheral bloodsmear, QBC and Antigen Detection in Malaria Diagnosis. Journal of Clinical and Diagnostic Research2011 October; Vol-5(5): 967-969.

47. Stephens J K, Phanart K, Rooney W, Barnish G. A comparison of three malaria diagnostic tests, under field conditions in North-west Thailand.Southeast Asian J Trop Med Public Health 1999; 30: 625–630.

48. Palmer, C. J., Lindo J F, Klaskala W I , Quesada J A , Kaminsky R, Baum M K , Ager A L. Evaluation of the optimal test for rapid diagnosis Plasmodium vivax and Plasmodium falciparum malaria . J. Clin. Microbiology1998 36:203–206.

49. Quitana, M., Piper R , H. Boling, Makler M, Sherman J E, Fernandez E, and Martin S . Malaria diagnosis by dipstick assay in a Honduran population with coendemic Plasmodium falciparum and vivax. Am. J. Trop. Med. Hyg 1998; 59:868–871.

50. Hunt-Cooke, A. H., Chiodini P L, Docherty T, Moody A H, Ries J, Pinder M. Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (optimal®) with microscopy for the detection of malaria parasites in human blood samples. Am. J. Trop. Med. Hyg 1999; 60:20–23.

51. Shiff C J, Minjas J, Premji Z.The parasight-F test: a simple rapid manual dipstick test to detect *Plasmodium falciparum* infection. Parasitol Today 1994;10*:* 494–495.

52. Iqbal, J., Hira P R, Sher A, A. A. Al-Enezi. Diagnosis of imported malaria by Plasmodium lactate dehydrogenase (pldh) and histidine-rich protein 2 (pfhrp-2)-based immunocapture assays. Am. J. Trop. Med. Hyg 2001;64:20–23.

53. Geoffrey Playford, John Walker. Evaluation of the ICT Malaria P.f/P.v and the optimal Rapid Diagnostic Tests for Malaria in Febrile Returned Travellers J. Clin. Microbiol. Nov 2002 vol. 40 :66-4171

54. Moody, A., A. Hunt-Cooke, Gabbett E, and Chiodini P. Performance of the optimal® malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London. Br. J. Haematol 2000; 109:891–894.

55. Fryauff, D. J., Purnomo, Sutamihardja M A, Elyazar I R S , Susanti I, Krisin, Subianto B, and Marwoto H. Performance of the Optimal assay for detection and identification of malaria infections

in asymptomatic residents of Irian Jaya, Indonesia. Am. J. Trop. Med. Hyg 2000; 63:139–145.

56. Malik S, Khan S, Das A, & Samantaray JC. Plasmodium lactate dehydrogenase assay to detect malarial parasites. Natl Med J India 2004;17 (5), 237-922.

57. Makler, M. T., J. M. Ries, J. A. Williams, J. E. Bancroft, R. C. Piper, B. L. Gibbins, and D. J. Hinrichs. 1993. Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. Am. J. Trop. Med. Hyg.48:739–741.

58. Oduola, A. M., Omitowoju G O, Sowunmi O, Makler M T, Falade C O, et al, *Plasmodium falciparum*: evaluation of lactate dehydrogenase in monitoring therapeutic response to standard anti-malarial drugs in Nigeria. Exp. Parasitology 1997; 87:283–289.

59. Piper, R. C., Vanderjagt D L, Holbrook J J, Makler M. Malaria lactate dehydrogenase: target for diagnosis and drug development. Ann. Trop. Med. Parasitology. 1996;90:433.

60. Nandwani S, Mathur M, and Rawat S. Evaluation of the direct acridine orange staining method and Q.B.C. test for diagnosis of malaria in Delhi, India. J Commun Dis 2003; 35 (4):279-82.

61. Pinto M J W, Rodrigues S R, Desouza R, and Verenkar M P.Usefulness of quantitative buffy coat blood parasite detection system in diagnosis of malaria. Indian Journal of Medical Microbiology 2001;19 (4), 219-221.

62. Urmila shenoi D.Evaluation of acridine orange stain of centrifuged parasites in malarial infection.Indian Journal of Medical Sciences1996;50(7):228-230.

63. Ahmad *et al*.,Thrombocytopenia in malaria. J coll Physicians surg Pak2009;19(11):708-710.

64. Jadhav U M ,Patkar V S ,Kadam N N.thrombocytopenia in malaria-correlation with type and severity of malaria.JAPI 2004 ; 52:615-618.

65. Bhushan Katira, Ira shah.thrombocytopenia in Plasmodium vivax infected children.J Vector Borne disease 2006; 43:147-149.

66. Alfonso J *et al*., anemia and thrombocytopenia in children with plasmodium vivax malaria. journal of Tropical Paediatrics 2005;52(1)49-51.

PROFORMA

Name :

Age /sex :

Address :

Inpatient Number :

Date of Admission :

Complaints	Yes	No
Fever chills ,rigor, sweat		
Duration & type of fever,		
Headache		
Muscle ache		
GIT symptoms(abdominal pain,anorexia, vomiting,diarrhoea)		
Altered sensorium		
Seizures		
Renal symptoms		
History suggesting other focus of infection		

General Examination

Pallor

Icterus

Pedal Edema

Cyanosis

Clubbing

Generalized lymphadenopathy

Systemic Examination

1 .Abdomen

Hepatomegaly

Splenomegaly

2 .CardioVascular System

3 .Respiratory System

4 .Central Nervous System Examination

Investigations:

Haemoglobin

Total count/differential count

Platelet count

Blood Sugar

Liver function tests

Sr.Bilirubin

SGOT/SGPT

Renal function tests

Urea,Creatinine

Sr.Electrolytes

Tests	Positive	Negative	Species
Peripheral smear examination			
Quantitative Buffy Coat			
Rapid Diagnostic Test(pLDH)			

ஆராய்ச்சி பற்றிய தகவல் அளிக்கப்பட்ட ஒப்புதல் படிவம்

---------- தகவல் அளிக்கப்பட்ட ஒப்புதல் படிவம் ---------- என்ற ஆண்/பெண் குழந்தையின் தாய்/தந்தை அல்லது உறவினராகிய ---------- என்ற என்னிடம் குழந்தைக்கு மலேரியா காய்ச்சலாக இருக்கலாம் என்று முழு உடல் பரிசோதனைக்குப் பிறகு மருத்துவரால் தெரிவிக்கப்பட்டது. இதனை உறுதி செய்யவும், மூன்று இரத்தப் பரிசோதனைகளில் சிறந்தது எது எனக் கண்டறியவும் இரத்தப் பரிசோதனைக்காக 3 ml இரத்த மாதிரிகள் கவனமாக, சுகாதாரமான முறையில் எடுக்கப்படும் என்று தெரிவிக்கப்பட்டது.

நோயின் தன்மையை பற்றியும், அதன் விளைவுகள் பற்றியும் மருத்துவரால் தெளிவாக எடுத்துரைக்கப்பட்டது. இந்த ஆய்வில் தேவைப்படும் பரிசோதனைகள் எழும்பூர் குழந்தைகள் நல மருத்துவமனையிலும் ஆய்வகராலும் பொருள் செலவின்றி செய்து தரப்படும் என்று எடுத்துரைக்கப்பட்டது.

இந்த ஆய்வு பற்றி எனக்கு விளக்கமாக எனது தாய்மொழியில் சொல்லப்பட்டது. இந்த ஆய்வின் பங்கெடுத்துக் கொள்வதாக எனது குழந்தைக்கு ஏற்படக்கூடிய அபாயங்கள் மற்றும் நன்மைகள் பற்றி எனக்கு விளக்கப்பட்டது. எனது குழந்தைக்கு வழக்கமாக செயல்படும் மருத்துவ கவனிப்பு அளிக்கப்படும் என்று தெரிவிக்கப்பட்டது.

இந்த ஆய்வின் எனது குழந்தையை பங்கெடுத்துக் கொள்ள முழுமனதுடன் சம்மதிக்கிறேன். கேள்விகள் கேட்டதற்கு எனக்கு வாய்ப்பளிக்கப்பட்டுள்ளது.

இந்த ஆய்வில் பங்கேற்பது தன்னிச்சையானது. எந்த நேரத்திலும் என் குழந்தை பங்கேற்பதை எந்த விளக்கமும் தராமல் நிறுத்திக் கொள்ளலாம். மற்றும் இதனால் என் குழந்தைக்கு கிடைக்க வேண்டிய மருத்துவ சிகிச்சைக்கு எந்த இடையூறும் ஏற்படாது என எனக்கு தெரிவிக்கப்பட்டது.

எனது குழந்தையிடம் இருந்து பெறப்பட்ட இரத்த மாதிரிகள் உயர் பரிசோதனை கூடங்களுக்கு அனுப்புவதற்கு சம்மதிக்கிறேன்.

இந்த ஆய்விலிருந்து கிடைக்கும் முடிவுகளை பயன்படுத்துவதை கட்டுப்படுத்தாமலிருக்க நான் சம்மதிக்கிறேன்.

குழந்தையின் பெற்றோர் / கண்காணிப்பாளர் கையெழுத்து – விரல் ரேகை.

பெயர் விலாசம்

சாட்சியின் கையெழுத்து

1.பெயர் / விலாசம்

2. பெயர்/விலாசம்

ஆய்வாளர் பெயர்

கையெழுத்து

தேதி

CONSENT FORM

I _____father/mother/guardian of _____aged _____ years, boy /girl was informed by the doctor that my child is suffering from fever and blood sample of about 3ml will be taken in aseptic conditions free of cost to confirm the diagnosis of malaria after clinical examination.

To arrive at a diagnosis of malaria and to compare the three different lab investigations (peripheral smear, Rapid Diagnostic Test, Quantitative Buffy Coat) has to done and the investigator will pay the required sum of money to get the special investigations.

I therefore agree to get my child participate in this study with my own knowledge and I will provide the correct information needed. There will be no objection from my side for my child's examination and investigations.

I_____ father/mother /guardian of the above mentioned child do hereby agree and allow my daughter /son/ to participate in the study.

I confirm that I have been told about this study in my mother tongue and have had the opportunity to ask questions.

I confirm that I have been told about the risks and potential benefits for my child's participation in the study.

I understand that my child's participation is voluntary and I have the right to withdraw my child from this study at any part of time without giving any reasons and without my child's benefit being affected.

I agree not to restrict the use of any data or results that may arise from this study.

Signature / Thumbprint of Parent / Guardian:

Name &Address of the Parent /Guardian:

Signature of the medical officer:

Witness Signature:

1. Name &Address of the witness

2. Name &Address of the witness

Principle Investigator

Address:

Date:

ABBREVIATIONS

QBC	-	Quantitative Buffy Coat
RDT	-	Rapid Diagnostic Test
ICT	-	Immuno Chromatographic Test
PCR	-	Polymerase Chain Reaction
pLDH	-	Plasmodium Lactate Dehydrogenase
HRP2	-	Histidine Rich Protein 2
GIT	-	GastroIntestinal Tract
WHO	-	World Health Organisation
PBS	-	Peripheral Blood Smear